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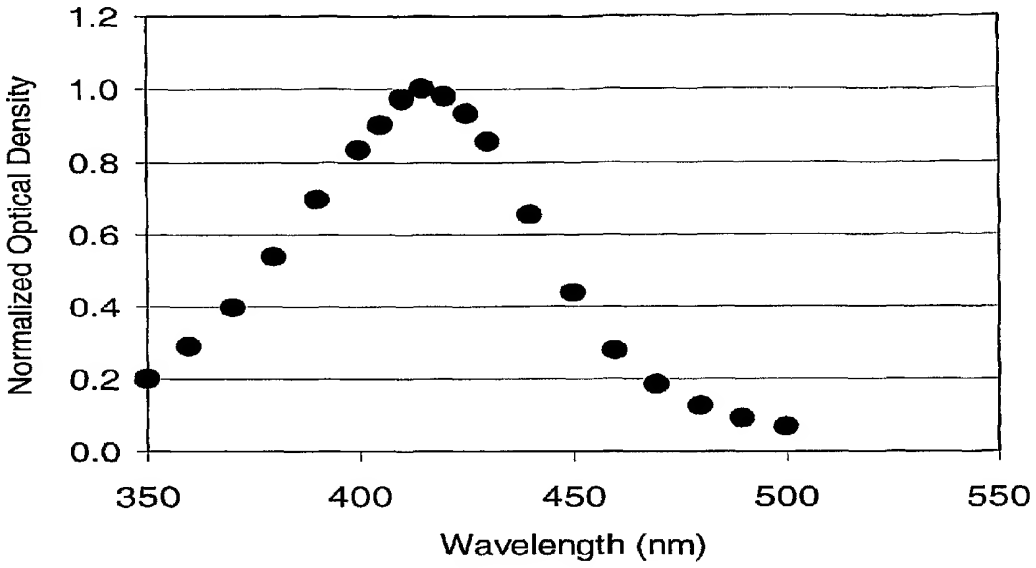
(54) **Title:** METHODS AND COMPOSITIONS FOR METAL NANOPARTICLE TREATED SURFACES



(57) **Abstract:** The present invention comprises methods and compositions comprising metal nanoparticles. The invention comprises metal nanoparticles and surfaces treated with a metal nanoparticle coating. The present invention further comprises compositions for preparing nanoparticles comprising at least one stabilizing agent, one or more metal compounds, at least one reducing agent and a solvent. In one aspect, the stabilizing agent comprises a surfactant or a polymer. The polymer may comprise polymers such as polyacrylamides, polyurethanes, and polyamides. In one aspect, the metal compound comprises a salt comprising a metal cation and an anion. The anion may comprise saccharinate derivatives, long chain fatty acids, and alkyl dicarboxylates.

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METHODS AND COMPOSITIONS FOR METAL NANOPARTICLE TREATED SURFACES

RELATED APPLICATIONS

This application claims the priority of U.S. Provisional Patent Applications No. 60/771,306, filed February 8, 2006 and 60/771,504, filed February 8, 2006, and is a continuation-in-part of U.S. Patent Application Serial No. 11/194,951 and PCT/US2005/27261, filed August 1, 2005,each of which claim the priority of U.S. Provisional Patent Application No. 60/592,687, filed August 1, 2004, each of which is herein incorporated in its entirety.

FIELD OF THE INVENTION

The invention relates to compositions comprising metal nanoparticles, their preparation, the application of the compositions to surfaces and methods of preparation.

BACKGROUND OF THE INVENTION

Silver, which is commonly used in jewelry, is also known for its antimicrobial properties and has found widespread use in biological and medical applications. A large number of commercial medical products with antimicrobial silver are used in wound care and other medical applications. Silver has high electrical conductivity (63.01x 10^6 S/m at 20° C) and thermal conductivity (429 W/m.K) which has led to its application in electrical, electronics and thermal transfer fields. In addition, silver has very high reflectivity and low emissivity and has found uses in adaptive optics and in making items such as optical mirrors and reflectors.

Silver has been used to make conductive elastomers. Such elastomers may be found as sheets or gaskets and are filled with up to 60% of a fine powder silver and such constructs have high conductivities. These elastomers typically can maintain their conductivities even after being stretched by 300%. An example of use of such products is a sheet form of a silver powder-filled elastomer applied to the surface of a large object, such as an airplane. The silver powder–filled sheet absorbs radio frequencies thus making the surface invisible to radar. Such silver-powder elastomeric covered objects are potentially useful in military applications. Covering surfaces with these materials adds considerable weight to the object because sixty percent of the weight of the covering is silver or other conductive metal.

One approach to making lighter conductive elastomers is to apply a metallic layer only on the surface. The layer or coating is applied by traditional methods such as electro-less plating

or vapor deposition. In general, coated fibers are not robust as the metal does not adhere well to the underlying elastomer substrate and often fail under even small strains. Coatings or layers of metals, such as silver, have been used on many types of fibers or other surfaces to render the surface antimicrobial or to resist growth of organisms, or to provide for a highly reflective surface. These coatings or layers often release metal, due to chemical or mechanical forces, and thus provide an unhealthy amount of metal to the environment or the surface fails to meet its intended use.

What is needed are methods and compositions for treating surfaces with metals, such as silver and others, so that the metal is retained on the surface and the surface is capable of meeting its intended usage for an extended time.

SUMMARY OF THE INVENTION

The present invention comprises metal nanoparticles, compositions comprising nanoparticles, such as stabilized silver nanoparticles, that are formed in a fluid environment and comprises methods of making and using these compositions. The nanoparticles and compostions of metal nanoparticles of the present invention generally comprise metal-containing nanoparticles in the size range of 0.1 to 100 nm with approximately 50 nm being the largest proportion of a size distribution of the nanoparticles.

The compositions of the present invention can be made with aqueous or non-aqueous solvents. The compositions of the present invention possess good shelf life and can be utilized in rendering surfaces with a coating of metal nanoparticles. Non-aqueous compositions may be based on solvents that have a range of boiling points from room temperature to above 300° C for some thermal transfer fluids. Non-aqueous metal nanoparticle compositions may be made by extracting the nanoparticles from aqueous compositions into a non-aqueous phase. As used herein, non-aqueous means organic media that are generally immiscible with water over large composition ranges as are generally understood by those skilled in the art. The amount of metal, such as silver, zinc, copper, gold, palladium, rhodium, or iridium, content in nanoparticle compositions can be adjusted by choosing the desired amount of metal in the preparation of the initial composition.

Differing amounts of metal loading (by amount of nanoparticles attached) on the surfaces can be achieved, for example, by successive multiple treatments or continued immersion of the treated object or surface in a uniform nanoparticle composition until the desired metal loading amount is reached. In general, the compositions are not viscous which

allows for ease in coating many preformed articles uniformly and thus rendering them metal treated. Often the techniques such as thermal evaporation or plasma deposition processes are unsuitable to achieve uniform deposition of metal, such as silver, inside objects with small ratio bores and long lengths because of the inherent concentration gradients. The compositions of the present invention easily coat or treat such surfaces, in addition to uniform and non-uniform surfaces, in part due to the low viscosity and low surface tension of a nanoparticles composition.

Materials which may be metal treated using the methods and compositions herein include, but are not limited to, catheters (venous, urinary, Foley or pain management or variations thereof), stents, abdominal plugs, feeding tubes, cotton gauzes, fibrous wound dressings (sheet and rope made of alginates, CMC or mixtures thereof, crosslinked or non-crosslinked cellulose), foam materials, collagen or protein matrices, hemostatic materials, adhesive films, contact lenses, lens cases, bandages, sutures, hernia meshes, mesh based wound coverings, ostomy and other wound products, hydrogels, creams, lotions, gels (water based or oil based), emulsions, liposomes, microspheres, ointments, adhesives, porous inorganic supports such as titania and those described in US 4,906,466, chitosan or chitin powders, metal based orthopedic implants, metal screws and plates, synthetic fabrics, nylon fibers, fabrics or its blends, and fabric fibers and woven and nonwoven materials, such as silk, rayon, wool, polyester, acrylic, acetate, Other surfaces, including dental and veterinary products and non-medical devices, made of silicone, polyurethanes, polyamides, acrylates, ceramics, thermoplastic and elastomeric materials may be treated with the nanoparticles compositions of present invention. The nanoparticles compositions of the present invention deposit nanoparticles on surfaces, and thus the surfaces that can be treated or coated by the present invention are not limited to those listed herein.

Nanoparticle compositions for different polymeric or metal surfaces that can be prepared from liquid compositions are also contemplated by the present invention. Such coating compositions can be hardened by solvent loss or cured by thermal or radiation exposure. Another aspect of the present invention comprise compositions comprising the nanoparticle compositions taught herein in combination with other active agents and antimicrobial agents such as glasses and zeolites similar to those disclosed in US 5,049,139 and US 6,248,342 which are incorporated by reference in their entirety.

Different methods are taught to treat the surfaces with nanoparticle compositions of the present invention. A method comprises making nanoparticle compositions comprising nanoparticles, contacting the nanoparticle composition and the surface or surfaces for a

sufficient period of time and rinsing the surface of the excess of the nanoparticle composition and drying the surface with nanoparticles adhered thereto. Several modifications of the disclosed method are possible without departing from the scope of the invention. Surfaces may also be treated with non-aqueous metal nanoparticle compositions.

Silver or other metal nanoparticles may be formed in situ on a surface,.. For instance, a method comprises providing a suspension comprising finely dispersed particles of a silver or metal compound in which a surface is immersed or contacts the suspension, followed by addition of a reducing agent for a specified period of time or until the silver or metal compound is reduced to nanoparticles, that are predominantly mono-disperse, and the nanoparticles attach or adhere to the surface.

The nanoparticle compositions of the present invention can be used in other compositions where an antimicrobial environment or antifouling environment is desired or where a reduction in microbial growth, or a reduction in odor would be useful. For example, the silver nanoparticles compositions may be added to paints, cosmetics, on wound dressings to control of odor from wound exudates, in dental compositions, in products used in bowel or vascular surgery, oral hygiene products, bathroom products, textile products, coatings, natural or synthetic polymers adhesives, paint products, polymer films, paper, leather, rubber and plastic articles. Unfinished and finished articles such as yarn or bolts of cloth may also be rendered antimicrobial.

Other applications for silver nanoparticle compositions of the present invention contemplated are in the catalysis of oxidation of olefins, in catalytic reduction of hydrogen peroxide, as polishing slurries, dissipation of static charge from surfaces, increasing thermal conductivity of liquids, increasing electrical conductivity, in the preparation of radio frequency or similar radiation shields, and in analytical chemistry for surface enhanced Raman spectroscopy.

The nanoparticle compositions of the present invention are made by relatively straightforward methods, are water or solvent based, possess long shelf life (nearly a year) and can be made in large volumes and thus, the production process is scalable. The components of the compositions are relatively non-hazardous and can be washed off from treated surfaces to leave behind the nanoparticles. The nanoparticle compositions may be optically clear, non-viscous and may be stored for long periods of time at room temperature, require no special storage conditions, are resistant to discoloration when exposed to light, are thermally stable,

fairly stable to acids and bases, and are able to withstand thermal cycling and conventional centrifugation.

The compositions of the present invention may comprise silver or other metal nanoparticles. The silver or metal compounds from which the nanoparticles of the present invention may comprise any type of anion, including inorganic or organic anions. Such anions may be organic, and include, but are not limited to, those taught in PCT Applications Serial Nos. PCT/US05/27260 and PCT/US05/27261 such as imidic organic anions, saccharine and saccharinates.

The nanoparticles of the present invention are made by combining a solvent, which may be water or a mixture of water and known miscible organic solvents, generally less than 35% v/v alcohol, a stabilizer which may be a polymer and/or a surfactant, a silver compound and a reducing agent. A surfactant capable of preventing agglomeration of the particles, such as a anionic, non-ionic or amphoteric surfactant. Known water miscible organic solvents include lower straight chain (C_1-C_6) or branched alcohols, acetone, tetrahydrofuran, formamide, dimethyl formamide, acetamide and other similar solvents. The reducing agent, which is thought to trigger the nanoparticle formation in solution, includes monomeric or polymeric organic chemical compounds comprising one or more electron donating groups with substituted or non-substituted nitrogen atoms, including but not limited to, triethanolamine and N,N,N',N' tetramethyl ethylene diamine (TEMED).

The aqueous silver nanoparticle compositions may be stabilized with a polymer. The polymer may be a homopolymer or copolymer and may be synthetic or natural and is usually water-soluble. Non-limiting examples of polymers are those comprising amide or substituted amides, primary, secondary or tertiary nitrogen, and urethane moiety in the main chain or side chains.

Treated surfaces take on a coloration that increases in intensity as more nanoparticles deposit. An aspect of the present invention comprises a method for creating a more whitened surface appearance for treated surfaces by applying to inanoparticle treated surface a hydrogen peroxide solution, washing off the solution, and drying the surface.

Antimicrobial silver compositions have utility not only in imparting an antimicrobial property to medical devices but can also reduce the odor causing bacteria, in items, including, but not limited to, hosiery products such as panty hose, socks, undergarments, swim wear products, outfits for hunters and trekkers, ski wear products, athletic wear products for a variety of sports, for disinfection purposes, it can be used in household or consumer products such as

bathroom or kitchen products, filters for humidifiers, shower curtains, cutting boards, sink sponges, bath sponges, and pumice stones. Compositions of the present invention can be also be used to treat a foam or porous matrix that can be added to unpotable water to disinfect it. In the construction industry, for the control of mold and mildew in homes the wooden structures during construction may be sprayed with the antimicrobial silver compositions of the present invention. Production of electrically conductive or reflective elastomeric materials is made by causing nanoparticles of the present invention to adhere to such elastomeric materials.

The present invention also contemplates use of radioactive metals (for example $^{110m}Ag^{+}$) compositions and their methods of preparation and their uses, for example, in articles that may be used as tracers. The nanoparticle compositions of the present invention can also be the starting material for producing dry nanoparticle powders suitable for many uses in material science and metallurgical applications.

BRIEF DESCRIPTION OF FIGURES

Figure 1 shows a representative spectrogram obtained by UV-Visible spectroscopic analysis of an aqueous silver nanoparticle composition in accordance with the present invention.

Figure 2 shows a representative spectrogram obtained by UV-Visible spectroscopic analysis of a non-aqueous silver nanoparticle composition in accordance with the present invention, wherein the solvent comprises chloroform.

Figure 3 shows a representative transmission electron micrograph of an aqueous silver nanoparticle composition in accordance with the present invention.

Figure 4 shows the particle size distribution of an aqueous silver nanoparticle composition in accordance with the present invention.

Figure 5 shows a representative transmission electron micrograph of a non-aqueous silver nanoparticle composition in accordance with the present invention, wherein the solvent comprises chloroform.

Figure 6 shows the particle size distribution of a non-aqueous silver nanoparticles composition in accordance with the present invention, wherein the solvent comprises chloroform.

Figure 7 shows representative spectrograms obtained by UV-Visible spectroscopic analysis of an aqueous silver nanoparticle composition in accordance with the present invention, wherein, as indicated in the figure, the aqueous silver nanoparticle composition was either prepared fresh (4 h) or analyzed at after storage at about 25° C for about 11 months.

Figure 8 shows representative spectrograms obtained by UV-Visible spectroscopic analysis of various aqueous silver nanoparticle compositions in accordance with the present invention which were prepared from various sodium salts.

Figure 9 shows representative spectrograms obtained by UV-Visible spectroscopic analysis of various aqueous silver nanoparticle compositions in accordance with the present invention which were prepared from various sodium salts, wherein the various aqueous silver nanoparticle compositions comprise the anion indicated.

Figure 10 shows representative spectrograms obtained by UV-Visible spectroscopic analysis of various aqueous silver nanoparticle compositions in accordance with the present invention which were prepared from various sodium salts, wherein the various aqueous silver nanoparticle compositions comprise Tween 20 (CAS No. 9005-64-5; $C_{58}H_{114}O_{26}$; known alternatively as polyoxyethylene (20) sorbitan monolaurate) at the indicated concentrations (g/L).

Figure 11 shows representative spectrograms obtained by UV-Visible spectroscopic analysis of various aqueous silver nanoparticle compositions in accordance with the present invention, wherein the various aqueous silver nanoparticle compositions were prepared from solutions comprising silver nitrate at a fixed concentration of 0.1 M and sodium saccharinate at concentrations as indicated.

Figure 12 shows representative spectrograms obtained by UV-Visible spectroscopic analysis of various aqueous silver nanoparticle compositions in accordance with the present invention, wherein the aqueous silver nanoparticle compositions were prepared from solutions comprising silver nitrate at concentrations as indicated.

Figure 13 shows representative spectrograms obtained by UV-Visible spectroscopic analysis of aqueous silver nanoparticle compositions in accordance with the present invention, wherein the aqueous silver nanoparticle compositions were prepared from solutions comprising TEMED (CAS No. 110-18-9; $C_6H_{16}N_2$; known alternatively as N,N,N',N'-Tetramethylethylenediamine) added in the volumes indicated.

Figure 14 shows representative spectrograms obtained by UV-Visible spectroscopic analysis of aqueous silver nanoparticle compositions in accordance with the present invention, wherein the aqueous silver nanoparticle compositions were prepared by reverse addition from solutions comprising addition of silver nitrate in the volumes indicated.

Figure 15 shows representative spectrograms obtained by UV-Visible spectroscopic analysis of a non-aqueous silver nanoparticle composition in accordance with the present

invention, wherein, the solvent comprised chloroform and as indicated in the figure, the non-aqueous antimicrobial silver nanoparticle composition was either prepared fresh (4 h) or analyzed at after storage at about 25°C for about 3 months.

Figure 16 shows a representative experiment measuring the release of non-radioactive ("normal") and radioactive silver from a nylon surface comprising an antimicrobial silver nanoparticles composition in accordance with the present invention.

Figure 17 shows representative results obtained for testing relative biofilm formation on nylon tubing samples comprising an antimicrobial silver nanoparticles composition in accordance with the present invention.

Figure 18 shows representative spectrograms obtained by UV-Visible spectroscopic analysis of an aqueous antimicrobial silver nanoparticle composition in accordance with the present invention, wherein various aqueous antimicrobial silver nanoparticles compositions were prepared from solutions comprising various surfactants as indicated.

DETAILED DESCRIPTION OF THE INVENTION

The present invention comprises metal nanoparticles and compositions comprising metal nanoparticles and methods for making and using such compositions. The compositions comprising nanoparticles may comprise aqueous solutions or non-aqueous solutions. The nanoparticles of the compositions are generally uniform in size, generally spherical, and can be preformed or made in situ. Methods for using the compositions include, but are not limited to providing antimicrobial characteristics to surfaces, compositions and materials; providing odor control to compositions and materials, preparation of silver- or metal-coated surfaces, metal coating of flexible or elastomeric surfaces, anti-fouling coatings of metal nanoparticles for surfaces, preparation of ultra-smooth surfaces that are metal nanoparticle coated, surfaces or articles that are reflective and/or conductive due to the presence of the metal nanoparticles, and for use in manufacturing and other applications. An aspect of the invention is to provide medical devices that are antimicrobial for an extended period of time and to provide methods for coating or treating medical devices and materials to render them antimicrobial, and to provide a range of amounts of silver to surfaces. Use of the metal silver, as an example for metal nanoparticles of the present invention, is not intended to be limiting to the metal nanoparticles taught and claimed herein, and other metals can be used including, but not limited to, silver, copper, zinc, gold, platinum, rhodium, iridium and palladium, to form nanoparticles with an average size $\leq$50 nm in diameter that are generally spherical.

The nanoparticle compositions of the present invention are made from chemicals that are relatively non-hazardous. The metal nanoparticle compositions of the present invention may be water based and prepared by a wet process. Unlike the thermal evaporation and other vacuum based processes that produce dry silver nano-powders, the wet process produces silver nanoparticles that stay in solution, unlike dry powders that may be a dust hazard risk. As taught herein, the nanoparticles may be made with a metal, and for ease of reference, these metal nanoparticles are often referred to as silver nanoparticles. This reference is in no way is a limitation of the nanoparticles taught herein and all metals which function to make nanoparticles in the methods taught herein are contemplated by the present invention.

A nanoparticle composition of the present invention comprises metal, including but not limited to silver, copper, zinc, gold, platinum, rhodium, iridium and palladium nanoparticles with an average size $\leq$50 nm in diameter that are generally spherical and having relatively narrow particle size distribution. Although most particles are spherical other types of shapes can also form and be present in the compositions of the present invention.

Upon nanoparticle formation, the metal nanoparticles may impart a characteristic color to the treated surface or article. For example, silver nanoparticles impart a characteristic yellow to yellow amber color, depending on the concentration of nanoparticles present. When examined by UV-VIS spectroscopy a silver nanoparticle compositions yielded a characteristic spectrum (Figure 1) having a wavelength maximum around 420-425 nm. According to the physics of nanoparticles, the color is due to the plasmon resonance band associated with spherical silver nanoparticles having size of 5 to 10 nm. Even after increasing the starting concentration of silver, the peak value of 420-425 nm remains unchanged. This suggests that the average particle size obtained in the compositions is relatively independent of the starting concentration of the silver nanoparticles. With an increase in nanoparticle size the absorption peaks tend to red shift to a higher wavelength. The type of stabilizing agent used may also affect the wavelength maximum and the average particle size and the distribution. In the case of a composition stabilized by polyacrylamide, the wavelength maximum at 445 nm suggests that average nanoparticles size is somewhat larger than the composition stabilized by Polysorbate 20. The nanoparticle compositions of the present invention generally show only a single peak under UV-VIS spectroscopy.

Using the formula below, on a unit mass basis, one can calculate the available surface area of an example of silver nanoparticles of the present invention

$$\text{Surface Area} = 6/[\text{density} \times \text{particle dia}]$$

The available surface area per unit gram for a 15 nm diameter particles is 3.81e5 per cm^2/gm. The surface area for other nanoparticles of the present invention can easily be determined.

Non-aqueous compositions are contemplated by the present invention. By non-aqueous it is meant that the solvent component of the nanoparticle composition is non-aqueous, as in organic solvents, those that are not miscible with water such as chlorinated alkanes, esters of carboxylic acids (ethyl acetate, butyl acetate), esters of ethylene glycol, propylene glycol, toluene, xylene, lower alkenes, and this list is not exhaustive. Generally, non-aqueous solvents are non-polar in nature, though small amounts of water may be present. Even when solvents are immiscible with water they will have some finite solubility in water and similarly water will have a finite solubility in the organic solvent. Generally, dissolved water in an organic solvent will be less than 5% v/v. The non-aqueous solvents may be neat or may be binary or multi-component mixtures. For example, a solvent may be pure chloroform or it may be a mixture of chloroform and ethyl acetate (a binary mixture) or it can be a mixture of chloroform, ethyl acetate and toluene (ternary or multi-component mixture). Further, a solvent may be polar (aprotic or protic) or non-polar. They are useful in applications where aqueous silver compositions cannot be used. Non-aqueous compositions may be based on solvents that have a range of boiling points from room temperature to above 300° C for some thermal transfer fluids.

An example of a non-aqueous composition comprises chloroform as solvent. Figure 2 shows the UV-VIS spectrum of such a composition with a maximum peak ~ 430-435 nm, a slight red shift in spectrum in comparison to an aqueous composition occurs. In all other respects, the spectrum is identical to that for an aqueous composition. The small red shift of the absorption peak (< 5 nm) have previously been reported in published literature (Wang et.al., Langmuir, Vol. 14, pp 602 (1998)). However it is not attributed to an increase average size of silver nanoparticles but more likely a result of changes in polarity of the solvent that may shift the plasmon resonance band to the right. Further a spontaneous change in particle size is also not possible simply as a result of the extraction operation to draw silver nanoparticles from aqueous phase into the non-aqueous phase.

A TEM micrograph of silver nanoparticles is presented in Figure 3. The majority of silver nanoparticles in the compositions of the present inventions are generally close to spherical though occasionally some flat faces may be present. The silver nanoparticles shown were prepared in aqueous medium utilizing Polysorbate 20, silver saccharinate and TEMED. By measuring the diameter of at least 100 particles in the TEM image, an estimate of size

distribution of the silver nanoparticles was obtained. The corresponding particle size distribution of silver nanoparticles in aqueous medium is presented in Figure 4 and shows an average size of ~ 15 nm. Figure 5 shows TEM image of silver nanoparticles from a non-aqueous composition. The nanoparticles were first prepared in aqueous medium and then extracted into a non-aqueous solvent, chloroform. A few drops of chloroform solution comprising silver nanoparticles were dried on a standard copper grid. The majority of silver nanoparticles in the compositions of the present inventions are generally close to spherical. Figure 6 shows the size distribution of silver nanoparticles in a non-aqueous medium with an average size approximately 11-12 nm with all particles smaller than 25 nm. The average size of silver nanoparticles in a non-aqueous composition is quite close to the average size in an aqueous medium. This fact is not surprising when it is noted that the silver nanoparticles in the non-aqueous medium were extracted from the aqueous solution.

To be commercially feasible, the antimicrobial compositions of the present invention must exhibit reasonable shelf life. Figure 7 compares the UV-VIS spectra of an aqueous composition made fresh and after aging the composition at ambient temperature (25° C) for nearly a year. There is almost no difference between the two, suggesting no change in the particles size or particle size distribution. The data clearly demonstrate that the aqueous compositions of the present invention possess excellent shelf life

Long term shelf life is not limited only to the aqueous compositions of the present invention but extend to non-aqueous compositions as well. The non-aqueous composition was tested in chloroform for over 3 months by UV-VIS spectroscopy and found no change in the spectrum shape or peak wavelength.

In addition to uses in rendering medical and non-medical articles antimicrobial, both the aqueous and non-aqueous silver nanoparticles compositions can be used to impart antimicrobial properties to fluid based compositions. Non-limiting examples of fluid compositions include adhesives, household sprays, disinfecting solutions or compositions such as those disclosed in US 4,915,955 and incorporated by reference herein its entirety, coating compositions for indoor and outdoor wood products, and personal lubricants.

The nanoparticle compositions of the present invention may comprise a wide range of amounts of silver or other metals, referred to as silver or metal loading. Different amounts of silver in the compositions can be achieved simply by using the desired amounts of silver compounds during the production. For example, it would be logical to expect a larger amount of silver nanoparticle deposition when untreated articles or surfaces are treated with nanoparticle

compositions comprising a higher number of silver nanoparticles and vice versa. Alternately, an incremental amount of silver loading on a silver treated surface can be achieved by a secondary treatment using a silver composition having a lower amount of silver. Using nanoparticle composition having a particular silver amount, one can spray or dip an article or surface multiple times to effect higher silver loading on the article. Each successive dip or spray would cause an incremental increase in silver loading until the desired level is achieved. The nanoparticle compositions of the present invention are generally non-viscous or have low viscosities and allow for uniform coating or contacting of surfaces, particular surfaces micron sized features and rendering them antimicrobial or functional for other purposes.

Silver or metal content of nanoparticle compositions can be adjusted by a variety of methods. One can initially select the desired amount of the metal compound or dilute a nanoparticle composition having a known amount of metal nanoparticles. The diluent added may comprise water and may or may not comprise other components such as surfactant or other miscible solvents. The metal content may be increased by concentrating the nanoparticle compositions by removal of solvent by means known to those ordinarily skilled in the art. One can remove most of the solvent from the nanoparticle composition, and re-dilute to regenerate the nanoparticle composition to a different volume or the original volume, without causing the nanoparticles to agglomerate.

The metal nanoparticles of the present invention are formed from weakly water soluble silver compounds formed with a variety of anions both inorganic and organic. However, even highly water-soluble compounds may be used in the practice of the present invention. Metal compounds with imidic organic anions are useful, and though many examples are given with silver saccharinate, the invention comprises any metal compound that will form nanoparticles in the methods disclosed herein. Metal compounds having imidic organic anions are the subject of PCT/US2005/27260 incorporated by reference herein in its entirety, and all the compounds taught therein are included in the present invention. Metal compounds with derivatives of saccharin can be suitably employed. Other metal compounds, made by the reaction of soluble metal salts with compounds with active methylene groups e.g. acetylacetonate and derivatives may also be used.

In one embodiment of the invention, antimicrobial compounds comprise compounds of silver as represented by:

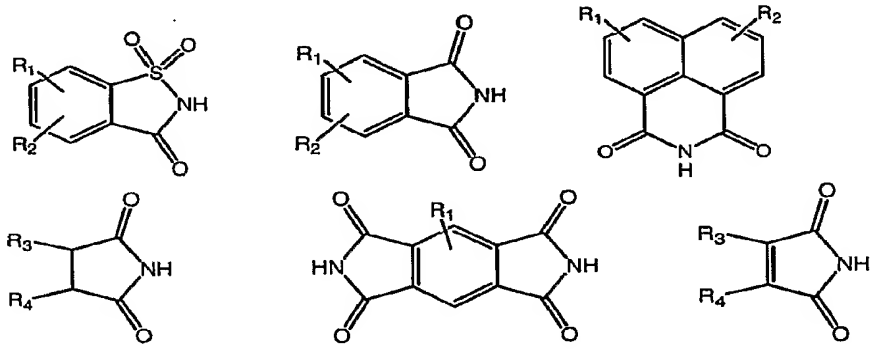
$M^{+}X_{(n)}$ wherein,M is a metal, such as silver, zinc copper, platinum, rhodium, iridium or palladium, n is 1 or more X is selected from A, B or C where R_1 and R_2 are–P or –WP; and

W is a linker of branched alkyl chain of 1-27 carbon atoms, straight alkyl chain of 1-27 carbon atoms, monoethers containing 2-20 carbon atoms and polyethers containing 2-20 carbon atoms; and

P is hydrogen, halogen atoms, haloalkyl, amide, sulfate, phosphate, quarternary ammonium, hydroxyl, hydroxymethyl, phosphonate, amino, carboxyl, carboxymethyl, carbonyl, acetyl, succinimidyl ester, isothiocyanate, isocyanate, iodoacetamide, maleimide, sulfonyl halide, phosphoramidite, alkylimidate, arylimidate, acide halide, substituted hydrazines, substituted hydroxylamines, carbodiimides, cyano, nitro, fluormethyl, nitrophenyl, sulfonamide, alkenyl or alkynyl; and

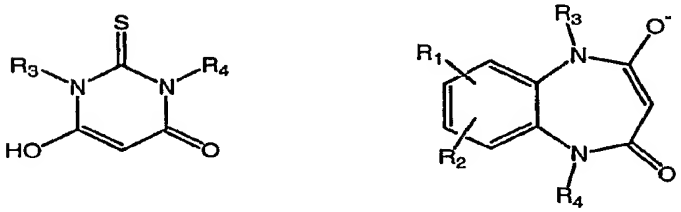
R_3 and R_4 are hydrogen, straight alkyl with C_1-C_8 carbon atoms, optionally terminating in aryl or substituted aryl groups, branched alkyl with C_1-C_8 carbon atoms, phenyl, substituted phenyl, benzyl, substituted benzyl and fluoromethyl; and

A is one of the following:



and

B is one of the following



R_1 and R_2 are –P and –WP as described above, and

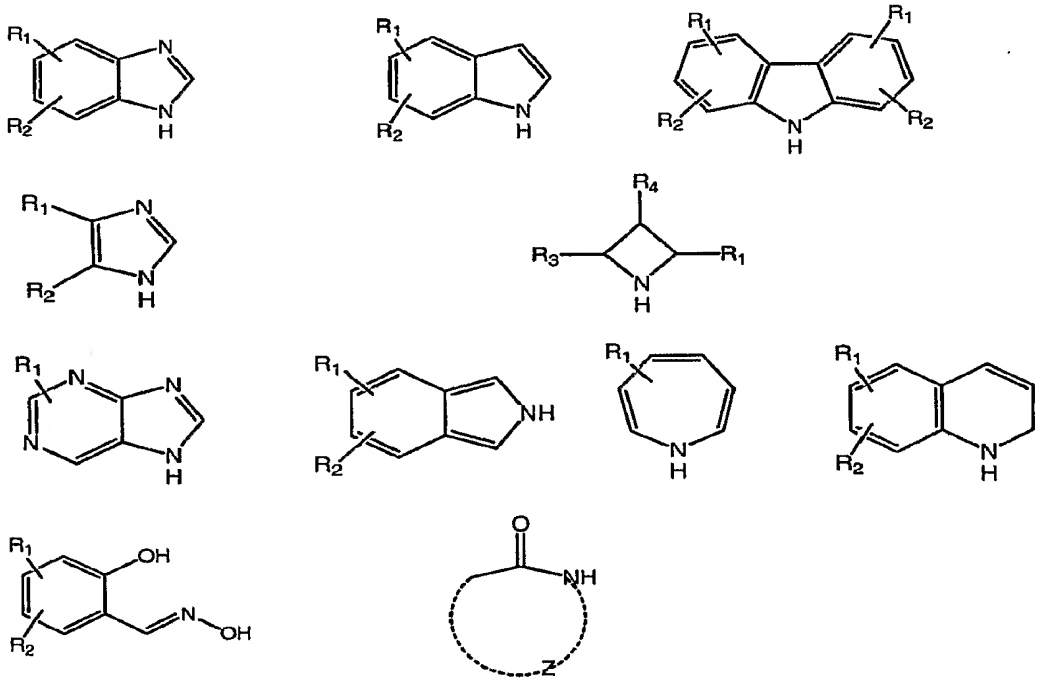
W is a linker as described above, and R_3 and R_4 are as described above.

C = behenate or bis (2-ethylhexyl) sulfosuccinate

Another embodiment of the invention comprises complexes of silver

$$M^{+}[Y^{-}]_{n}$$

where M is a metal, such as silver, zinc copper, platinum, rhodium, iridium or palladium,, n is 1 or more; and Y is the following:

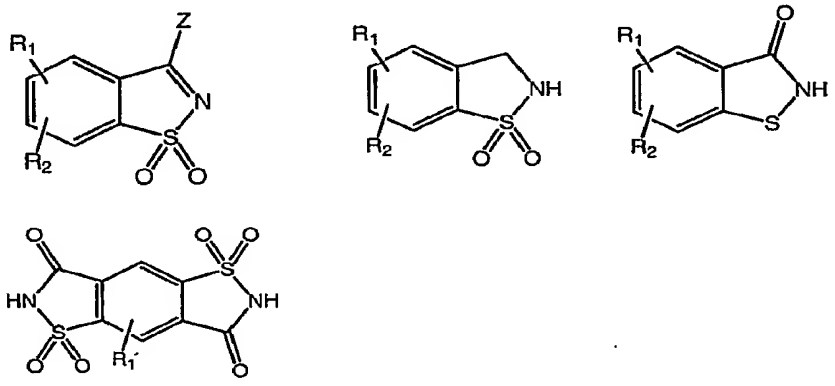


where R_1 and R_2 are selected from the group consisting of –P and –WP; as described above, and W is a linker as described above. R_3 and R_4 are described above and Z is C6 or C8 alkyl.

Another embodiment of the present invention comprises the following where

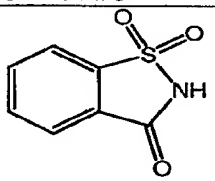
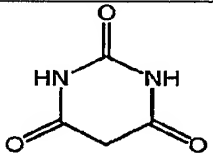
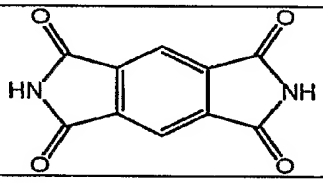
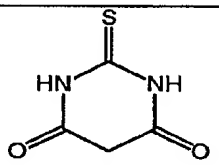
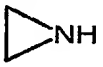
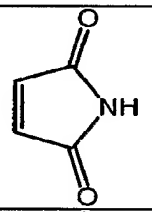
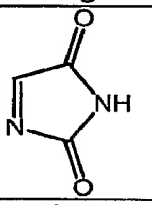
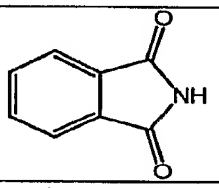
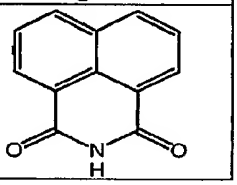
$M^{+}[Y'^{-}]n$

where M is a metal, such as silver, zinc copper, platinum, rhodium, iridium or palladium, N is 1 or more and Y'- is the following:



where R_1 and R_2 are selected from the group consisting of –P and –WP; as described above, and W is a linker as described above. R_3 and R_4 are described above and Z is amino, alkylamino, chloro, or HNX, wherein X in HNX comprises aryl, hydroxyl, amino, NHC_6H_5, or $NHCONH_2$. Other ligands that form silver compounds of the present invention comprise the following shown in Table 1:

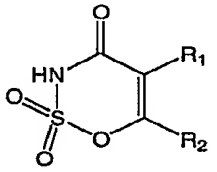
TABLE 1

ID	Name	Structure	ID	Name	Structure
1.01	1,1-Dioxo-1,2-dihydro-1λ⁶-benzo[α]isothiazol-3-one		1.06	Pyrimidine-2,4,6-trione	
1.02	Pyrrolo[3,4-*f*]isoindole-1,3,5,7-tetraone		1.07	2-Thioxo-dihydro-pyrimidine-4,6-dione	
1.03	Aziridine		1.08	Pyrrole-2,5-dione	
1.04	Azetidine		1.09	Imidazole-2,4-dione	
1.05	Isoindole-1,3-dione		1.10	Benzo[*de*]isoquinoline-1,3-dione	

The nanoparticles may be made from a single silver compound or mixtures of silver compounds. For example, a mixture might comprise silver compounds having high and low water solubilities. Further the binary mixture might comprise a range of 0 to 100% the weakly water-soluble silver compound. For example, when preparing silver nanoparticles, sodium saccharinate may be added to only 80% of the amount required to react with silver nitrate, then add TEMED and so on. Therefore in the mixture, there is silver nitrate (soluble salt) and silver

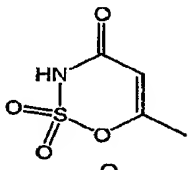
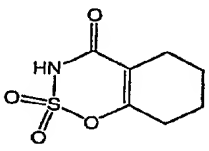
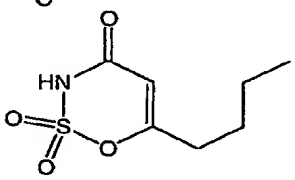
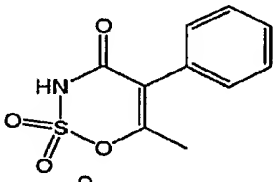
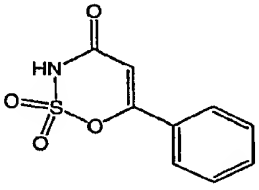
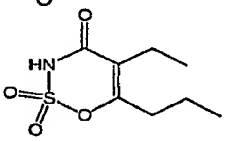
saccharinate (weakly soluble salt) together. Similarly one can weigh out powder forms of silver nitrate and silver propionate in any desired proportions (0% silver nitrate to 100%).

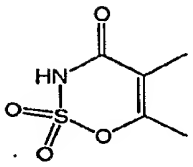
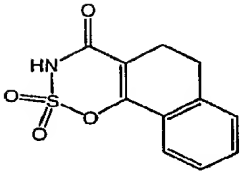
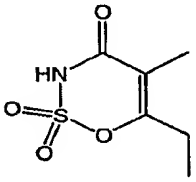
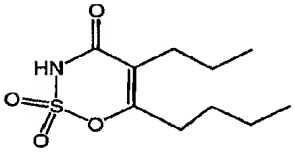
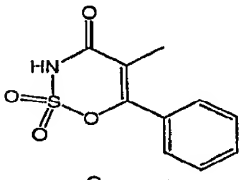
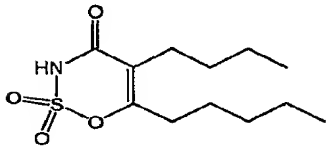
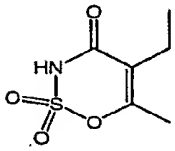
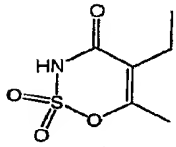
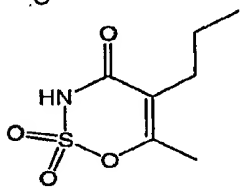
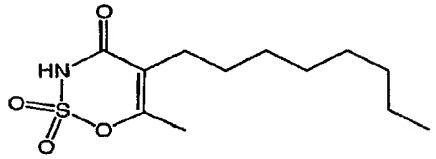
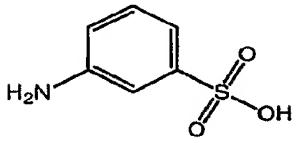
Metal compounds for use in compositions or devices of the present invention wherein the compound is X+Y-, wherein X is a metal, such as silver, zinc copper, platinum, rhodium, iridium or palladium, and Y is acesulfame, or derivatives thereof.



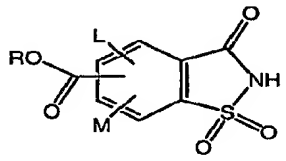
R_1 and R_2 are a hydrogen atom, optionally a branched alkyl group having from one to 20, or up to 10 carbon atoms, an aromatic hydrocarbon radical having up to 10 carbon atoms, or an aliphatic acyl radical having from two to four carbon atoms, R_2 is an optionally branched alkyl group having up to 20 carbon atoms, or up to 10 carbon atoms, or an aromatic hydrocarbon radical having up to 10 carbon atoms, and in which R_1 and R_2 may also be linked to form an isocyclic ring which optionally may be substituted by further hydrocarbon radicals...Also included are the salts of the compounds of this formula. Additional compounds are shown in Table 2.

TABLE 2

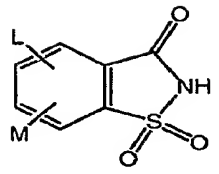
Name	Structure	Name	Structure
3,4-dihydro-6-methyl-1,2,3-oxathiazin-4-one-2,2-dioxide		3,4-dihydro-5,6-tetramethylene-1,2,3-oxathiazin-4-one-2,2-dioxide	
3,4-dihydro-6-n-butyl-1,2,3-oxathiazin-4-one-2,2-dioxide		3,4-dihydro-5-phenyl-6-methyl-1,2,3-oxathiazin-4-one-2,2-dioxide	
3,4-dihydro-6-phenyl-1,2,3-oxathiazin-4-one-2,2-dioxide		3,4-dihydro-5-ethyl-6-n-propyl-1,2,3-oxathiazin-4-one-2,2-dioxide	

Name	Structure	Name	Structure
3,4-dihydro-5,6-dimethyl-1,2,3-oxathiazin-4-one-2,2-dioxide		3,4-dihydro-5,6-[2,1-(3,4-dihydro-)naptho]-1,2,3-oxathiazin-4-one-2,2-dioxide	
3,4-dihydro-5-methyl-6-ethyl-1,2,3-oxathiazin-4-one-2,2-dioxide		3,4-dihydro-5-n-propyl-6-n-butyl-1,2,3-oxathiazin-4-one-2,2-dioxide	
3,4-dihydro-5-methyl-6-phenyl-1,2,3-oxathiazin-4-one-2,2-dioxide		3,4-dihydro-5-n-butyl-6-n-amyl-1,2,3-oxathiazin-4-one-2,2-dioxide	
3,4-dihydro-5-ethyl-6-methyl-1,2,3-oxathiazin-4-one-2,2-dioxide		3,4-dihydro-5-isopropyl-6-methyl-1,2,3-oxathiazin-4-one-2,2-dioxide	
3,4-dihydro-5-n-propyl-6-methyl-1,2,3-oxathiazin-4-one-2,2-dioxide		3,4-dihydro-5-n-octyl-6-methyl-1,2,3-oxathiazin-4-one-2,2-dioxide	
3-Amino-benzenesulfonic acid			

The present invention comprises metal compounds comprising a metal and saccharincarboxylic acids or saccharincarboxylic acid esters of the formula: (I)



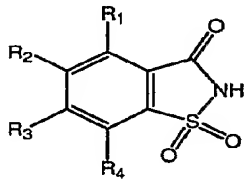
wherein the substituents have the following meanings: L and M are hydrogen, alkyl, alkoxy, cyano, alkylsulfonyl, nitro, trifluoromethyl and chlorine; and, R is H or alkyl with 1-6 carbon atoms. The present invention further relates to derivatives of saccharin of the formula:



(II)

wherein the L and M are independently selected from hydrogen, alkyl, alkoxy, cyano, alkylsulfonyl, nitro, trifluoromethyl and chlorine.

The present invention comprises metal compounds comprising a metal and derivatives of saccharin of the formula:



(III)

wherein R_1, R_2, R_3 and R_4 are independently selected from hydrogen, alkyl, alkoxy, cyano, alkylsulfonyl, nitro, trifluoromethyl and chlorine.

The compositions of the present invention comprise a solvent, and the solvent may be water or a mixture of water and known miscible organic solvents, a stabilizing agent which may be a polymer and/or a surfactant, a metal compounds such as a silver compound and a reducing agent. The solvent may be water or a mixture. If the solvent is a mixture where the water content may range between 55% v/v and 95% v/v, the mixture may be any water miscible organic solvents including, but not limited to, lower straight chain (C_1-C_6) or branched alcohols, acetone, tetrahydrofuran, formamide, dimethyl formamide, acetamide and other similar solvents. If the stabilizing agent used is a surfactant, surfactants including, but not limited to, polysorbates or Tweens, are useful. Any suitable surfactant may be used. The reducing agent, the agent that is thought to trigger the formation of silver nanoparticles in the solution includes, but is not limited to, tertiary, secondary and primary amines, tertiary, secondary and primary diamines, homopolymers or copolymers having primary amine, secondary amine and tertiary amine moieties. Amine compounds may be aliphatic or aromatic. Likewise, aliphatic and aromatic primary and substituted amides and polymeric amide analogs also can be used. An aromatic amide such as diethyl toluamide known as DEET also can be used. Other reducing agents are triethanolamine and N, N, N', N' tetramethyl ethylene diamine (TEMED). Polymeric compounds having TEMED moiety or other amines in the pendant chain or in the main chain may also be used as reducing agent.

The stabilizing agent may be a polymer, and a surfactant may or may not be used in addition to the polymer. The polymer may be a homopolymer or copolymer and can be synthetic or naturally derived. Non-limiting examples of polymers or copolymer suitable for use as stabilizers in the compositions include polymers formed from acrylamide and its derivatives, methacrylamide and its derivatives, polyamides, polyurethanes, polymers having no particular backbone but with urethane segments or tertiary amine groups in the side chains, other polymers predominantly polar in nature or co-polymers having a portion that is derived from polar co-monomers. Examples include, but are not limited to, acrylamide, methacrylamide, substituted acrylamides (i.e. $-CONH_2$ is replaced by $CON(R1)_2$, substituted methacrylamides, acrylic acid, methacrylic acid, hydroxyethyl methacrylate, acrylonitrile, 2-acrylamido-2-methylpropane sulfonic acid and its salts (sodium, potassium, ammonium), 2-vinyl pyrrolidone, 2-vinyl oxazoline, vinyl acetate, maleic anhydride and others. Though not wishing to be bound by any particular belief, it is believed that stability is achieved by steric hindrance due to the presence of polymer chains in such a way that the particle agglomeration and growth is suppressed.

The nanoparticle compositions of the present invention are fairly stable at low pH as well as high pH. The acids that can be added to antimicrobial silver compositions are organic acids including polymeric analogs such as polyacrylic acid, acetic acid, citric acid and similar acids though adding nitric acid > 10% will destroy the compositions by dissolving the silver nanoparticles. Nitric acid at concentration below 10% will also destroy the compositions over time. Adding 10% v/v ammonia solution does not affect the silver nanoparticle compositions (i.e. no color change is seen).

Silver content, as nanoparticles, of the compositions can be adjusted by initially selecting the starting amount of the silver compound in making the nanoparticles or by diluting the composition after making the nanoparticles. The optical density of the silver nanoparticles compositions obtained using low concentrations of silver salt may not reach 2.0. However, the optical density of compositions made with concentrated silver salt solutions may be extremely high requiring very high dilution (> 100 fold) for absorbance readings below 2. Just as nitric acid can destroy the silver nanoparticles compositions by dissolving, adding certain water miscible solvents causes nanoparticles to agglomerate and precipitate out. The silver content can be increased by concentrating the compositions by removal of solvent by means known to those ordinarily skilled in the art. In fact one can remove most of the solvent from the compositions, re-dilute to regenerate the composition to the original state without causing significant silver nanoparticle agglomeration.

The compositions of the present invention comprise silver nanoparticles and may also comprise weakly soluble silver compounds. In the course of the preparation of nanoparticles, a silver salt may formed in situ which may not be converted to silver nanoparticles during the reaction period. Silver compositions where the silver may or may not be present as unreacted trace of a salt are still encompassed by the present invention.

Another embodiment of the antimicrobial silver compositions of the present invention is a non-aqueous antimicrobial silver composition. Those skilled in the art have recognized that it is difficult to produce stable silver nanoparticles in a non-aqueous medium (Zeiri and Efrima, J. Phys. Chem., Vol. 96, pp5908-5917 (1992)). The non-aqueous silver nanoparticles compositions of the present invention may be prepared by extracting the nanoparticles from the aqueous compositions into a non-aqueous phase. While non-aqueous solutions containing silver have been made, the studies have not shown their antimicrobial efficacy. By non-aqueous we mean organic media that are generally immiscible with water over a large ratio between water and immiscible solvent. Non-aqueous solvents used in preparing the compositions of the present invention are methylene chloride, chloroform and other aliphatic and aromatic chlorinated solvents, cyclohexane, diethyl ether, ethyl acetate and mixtures thereof. The amount of silver content in non-aqueous compositions can be adjusted by choosing the proper amount of silver in the preparation of the aqueous composition followed by extraction of the aqueous composition and by further appropriate dilution if needed.

One embodiment of the present invention is compositions comprising the mixtures of a surfactant, a silver compound preferably a salt (that can ionize to a silver cation and an anion in solution), TEMED and water. These compositions are precursor compositions to the nanoparticle compositions of the present invention. Precursor compositions are then subjected to certain treatments to transform them into nanoparticle compositions of the present invention. For example, precursor compositions wherein the metal compound comprises silver can be heated to initiate the silver nanoparticles formation which is indicated by a yellow color. Heating can be achieved by direct or indirect contact with electric heating element, by IR lamps, by microwave energy, by acoustic energy or by the use of other electromagnetic radiation. Precursor compositions also may be converted to nanoparticle compositions by exposure to intense light energy (UV lamps, strobes, mercury vapor lamps, halogen lamps, laser beams etc). Precursor compositions may be employed to form silver nanoparticle compositions where the nanoparticles may take different shape and form. They may also be used in electroless plating applications in the preparation of silver coated reflective coatings on glass beads, plastic surfaces

for improving the light reflectance of signs at night, and other uses. Precursor compositions which are aqueous in nature may be made and stored below ambient temperature and used subsequently without any loss of performance.

Methods of Preparation of Nanoparticle Compositions

Different methods may be employed to prepare nanoparticle compositions of the present invention. An example of a silver nanoparticle preparation method comprises the following steps:

(i) preparing the aqueous solutions of a surfactant (and/or polymer), of sodium saccharinate (or a suitable anion) and of soluble silver salt solution,

(ii) adding a sodium salt solution to the surfactant solution under stirring,

(iii) further adding soluble silver salt solution to cause the precipitation of weakly soluble silver salt,

(iv) adding the tertiary diamine (TEMED) and,

(v) causing a temperature increase of the resulting solution and maintaining the increase for specific time period.

In another embodiment, after the temperature increase for a specific duration in step (v), the solution temperature is returned to room temperature. If desired, the solution temperature may also be lowered to a temperature other than room temperature. The temperature can be above or below the room temperature. ,The weakly soluble silver salt may not immediately form a clear precipitate, but this should not be considered as limiting the practice of the invention. A variation of the above method involves reversing the order of addition of sodium salt solution and soluble silver salt solution. A further variation involves substituting the surfactant with a water soluble polymer solution in step (i) with the other steps remaining the same, or reversing the sodium salt solution and the silver salt solution. The sodium salt solution and the silver salt solution can be added in no particular order.

In one embodiment using polyacrylamide as the stabilizer in one composition of the present invention, the preparation is as follows.

(a) preparing the polymer solution of desired concentration,

(b) adding in succession under mixing appropriate quantities of the alkali metal solution of appropriate anion such as saccharinate, soluble silver salt solution and the reducing agent and,

(c) causing a temperature increase and maintaining the temperature increase for a specified time period to form nanoparticles.

Optionally the solution may not be heated but left at room temperature under ambient light over a period of 24 hours to 7 days to complete the formation of silver nanoparticles. The temperature increase can be caused by methods known to those ordinarily skilled in the art. Alternately, light energy sources may be employed to form silver nanoparticles.

In preparing non-aqueous silver compositions of the present invention, a method comprises

(a) preparing the aqueous silver nanoparticles composition with desired silver content, as described herein;

(b) reducing its volume to concentrate the aqueous composition,

(c) extracting the said concentrate with non-aqueous solvent or solvent mixture and,

(d) recovering the non-aqueous solvent or solvent mixture comprising the extracted silver nanoparticles.

The step (b) above is optional especially if the silver content of the aqueous composition is significantly high. Likewise the step (c) optionally may be carried out multiple times, each time using a fresh portion of the non-aqueous medium. The temperature may be room temperature in the practice of this method of the present invention.

In the preparation of non-aqueous silver compositions of the present invention, one can optionally add to the non-aqueous solvent a compound that may be a liquid or a solid having at least one double bond in its molecular structure. For example one may add such a compound as an extraction aid in amounts up to 25% of the non-aqueous solvent to improve the extraction efficiency.

In an embodiment for making non-aqueous silver compositions, a double bond containing compound may also serve as a stabilizing agent in the preparation of the aqueous silver compositions. A double bond containing compound, such as an oleate or a sorbate, may be added instead of the surfactant. In the second case, one may form silver sorbate (in the presence of surfactant) and then convert the salt to nanoparticles using TEMED. The sorbate anion has two double bonds and the rationale is this organic anion may get readily transferred into the non-aqueous phase. Such a compound for example may be an oleate, sorbate, fumarate or cinnamate. The compounds listed by no means should be construed as limiting. The resulting aqueous silver compositions extract more readily with non-aqueous solvent transferring silver nanoparticles to the non-aqueous medium with greater efficiency and help to maintain the stability in non-aqueous environment.

A modification of the method of preparation of non-aqueous silver composition is to extract silver nanoparticles from aqueous silver compositions into a non-aqueous solution and then add a double bond compound to increase the stability of the compositions. One may add no more than 25% by weight of the non-aqueous solvent of this compound. Non-limiting examples of double bond compounds are oleic acid, sorbic acid, cinnamic acid and their derivatives. Polymeric compounds such as polyacetylenes, polyvinylenes and their derivatives can also be used that have some solubility in extracting the non-aqueous media.

Other compounds may be added to the compositions. For example, in some applications of non-aqueous compositions, long alkyl chain bearing thiols may be added to aid in the formation of metal nanoparticles layers on silicon and similar semi-conducting surfaces.

Effect of Process Conditions

Various parameters may affect the properties and performance of the compositions, such as silver compounds with different anions, the concentration effects of the silver salts, the stabilizing agent and the reducing agent. A robust process for producing silver nanoparticles can be used for nanoparticle deposition on various substrates.

Silver Salts with Different Anions

The antimicrobial silver compositions of the present invention are convenient to prepare. They were conveniently prepared starting from a variety of silver salts formed in-situ from corresponding sodium salts. Though one can also directly use silver salts in dry form if available without departing from the scope of the invention. The salts used may comprise organic or inorganic anions. The salts were then reduced to silver nanoparticles in the presence of a surfactant, Polysorbate 20, and TEMED by heating the resulting mixture in a microwave for a brief period. Stock solutions of Polysorbate 20 (~ 76 gm/L), silver nitrate (0.1M) and sodium salts (0.125M) were prepared and were used in a volume ratio of 1.2/4.0/3.0/1.2 for Tween® 20, sodium salt solution, silver nitrate solution and TEMED. UV/VIS spectra of silver nanoparticles compositions were measured on a Beckmann DU-20 spectrophotometer by diluting the composition with water (25 µl in 3 mL water) in a 1 cm path length cuvette. Deionized water was used as a reference.

Table 3 lists the sodium salts that were used in preparing corresponding silver salts in-situ. Of the 15 salts tested, only about half of them failed to form clear and stable yellow brown silver nanoparticles solution (Figure 8). Silver chloride (from sodium chloride) gave a red or flesh color precipitate that immediately settled at the tube bottom. In addition, silver salts with the following anions did not yield stable nanoparticles solutions: borate, tartarate, carbonate,

citrate, phosphate and lauryl sulfate though their spectra indicated a peak ~ 420 nm suggesting the formation of silver nanoparticles in size ~ 10 nm (Figure 9). Of the silver salt yielding solutions of poor stability, half were organic anions and the other half were inorganic suggesting the inability to form stable nanoparticles solutions was not related to their organic or inorganic nature. While the use of the silver salts of anions borate, tartarate, carbonate, citrate, phosphate and lauryl sulfate may not be optimal, their use in the preparation of antimicrobial compositions is encompassed by the present invention.

Table 3: Sodium salts with various inorganic & organic anions used in preparing silver nanoparticles compositions

Sodium salt type	Salt anion type	Precipitate or debris formed?	NP Solution Appearance
Chloride	Inorganic	Yes	Red, flesh color suspension, agglomeration
Borate	Inorganic	Yes	Dark green/grey suspension, agglomeration
Carbonate	Inorganic	Yes	Green/grey suspension, agglomeration
Sulfate	Inorganic	no, silver deposit on tube	Brown/yellow clear
Phosphate	Inorganic	yes	Grey clear, agglomeration
Acesulfame	Organic	no	Brown/yellow clear
Oxalate	Organic	no, silver deposit on tube	Brown/yellow clear
EDTA Di - salt	Organic	no	Brown clear
Tartarate	Organic	yes, some silver deposit	Green/grey suspension, agglomeration
Acetate	Organic	no, silver deposit on tube	Brown/yellow clear
Citrate	Organic	yes	Light green/beige suspension, agglomeration
Propionate	Organic	no, silver deposit on tube	Brown clear
Dioctyl sulfosuccinate	Organic	no, no silver deposit on tube	Brown clear
Lauryl Sulfate	Organic	yes	Grey/green suspension, agglomeration
Oleate	Organic	no, no silver deposit on tube	Brown clear

Note: The precipitate or debris are filtered off or centrifuged to prevent interference during UV/VIS spectral measurements

Another observation was the in situ formed salts that readily formed silver nanoparticles did not show any precipitate or debris formation. The embodiment that yielded no precipitate or debris comprises a method comprising the following steps of,

(i) preparing the aqueous solutions of the surfactant, sodium saccharinate (or a suitable anion) and silver salt solution,

(ii) adding the sodium salt solution and the tertiary diamine (TEMED) to the surfactant solution under stirring,

(iii) further adding soluble silver salt solution and,

(iv) causing a temperature increase of the resulting solution briefly and then returning the temperature to room temperature.

Therefore, the method of adding silver nitrate as the last ingredient in solution to previous ingredients is one embodiment of the present invention. Volume ratios of starting reagents of 1.2/4.0/3.0/1.2 for Tween® 20, sodium salt solution, silver nitrate solution and TEMED respectively are elements of an embodiment for making nanoparticles compositions.

Visually, the nanoparticle solutions prepared using sodium oleate was the best. There was no debris or any metallic silver deposits on the tube wall. This was somewhat expected because published work have reported on the beneficial effect of oleate on silver nanoparticles (Wang et.al., Langmuir, Vol. 14, pp 602 (1998)). The oleate stabilized nanoparticles solutions tend to be very stable. Stabilizing effect of oleate has been attributed to silver's interaction with pi electrons of the oleate double bond.

Figures 8 and 9 show plots of absorbance (normalized to OD = 1) versus wavelength for various organic and inorganic anions. The λ_{max} for inorganic anions is 415 nm (Figure 8) and their full width half maximum (FWHM) are of similar magnitude though the sulfate anion shows a tighter spectrum. Interestingly, the borate and carbonate anions project a spectrum that is similar to sulfate yet the nanoparticles solutions are not very stable. This indicates that under the conditions, the nanoparticles of small size ~ 10 nm and narrow distribution are formed with these two anions, but the ionic environment in those solutions is unable to prevent their agglomeration.

In comparison, silver nanoparticle solutions prepared from various organic anions more or less exhibit greater stability and the characteristic yellow brown color indicating presence of nanoparticles. Only a small difference in the spectral maximum among them is observed but with a wide variation in their spectra (Figure 9). For example, the solution with EDTA anion shows a peak OD at 390 nm and relatively sharp spectra. On the other hand, a tartarate based solution while having a peak at 415 nm reveals a practically flat spectra. Such spectra indicate a very broad silver particle distribution.

In Table 4 wavelengths are listed where peak OD was observed and FWHM values derived from the spectral data of solutions shown in the figures. Like inorganic anions we see λ_{max} around 415-425 nm for organic anions. The fact that we observed the same λ_{max} over so

many different anions suggests the mechanism of silver nanoparticle formation have little to do with the type of anions present. But, the agglomeration behavior suggests that the stability of silver nanoparticles formed very much depend on the anion type. Without being bound to any theory, the inventors are hypothesizing that the interaction of anions with silver nanoparticles if thermodynamically favorable yield stable solutions.

In the same table, the FWHM is listed for each spectrum. The number is a measure of the width of the spectrum. Smaller the FWHM number indicates sharpness of the spectrum. The FWHM value of 53 nm for EDTA anion is the smallest seen so far and that includes

Table 4: λ_{max} & FWHM values of UV-VIS spectra of silver nanoparticles compositions prepared with different anions

Salt anion	Anion type	λ_{max} (nm)	FWHM (nm) (full width half max)
Chloride	Inorganic	ND[+]	ND
Borate	Inorganic	415	90
Carbonate	Inorganic	415	92
Sulfate	Inorganic	415	65
Phosphate	Inorganic	ND	ND
Acesulfame	Organic	415	92
Oxalate	Organic	415	70
EDTA Di - salt	Organic	400	53
Tartarate	Organic	415	ND
Acetate	Organic	415	67
Citrate	Organic	ND	ND
Propionate	Organic	420	72
Dioctyl sulfosuccinate	Organic	425	66
Lauryl Sulfate	Organic	ND	ND
Oleate	Organic	420	91

ND = Not determined

published literature. The oleate FWHM value of 91 nm is fairly close to the value of 88 nm reported in a published paper that extensively examined oleate containing silver nanoparticle solutions prepared from silver nitrate. The present work differs from published accounts in that the FWHM values herein are for solutions made from silver salts with concentrations 10 to 100 times higher than those previously tested. The fact that similar FWHM was observed means practically no agglomeration of nanoparticles in the solutions occur even when using high silver concentrations.

Process Parameters

The effects of varying the stabilizer amount, reactants ratio, concentration of the reducing agent and the order of reagent addition on quality of the nanoparticle solutions were examined.

Appropriate stock solutions of sodium saccharinate, silver nitrate and Tween® 20 or Polysorbate 20 were prepared in de-ionized water. Reducing agent was used as received. Two methods to prepare silver nanoparticles were used. In Method A, a silver saccharinate suspension was first formed in the presence of surfactant by reacting silver nitrate and sodium saccharinate. To the suspension, TEMED was added and the resulting turbid mixture heated briefly in microwave oven to complete the nanoparticle formation. Method B involved mixing surfactant Tween 20, sodium saccharinate and TEMED in a capped vial to form a clear solution. Silver nitrate solution was added last and the vial contents heated in microwave oven to produce nanoparticles. In all experiments, microwave heating time was 10 seconds on medium setting (Oven Make: Quasar Instant Matic Cooking, 1500W).

Nanoparticle solutions were characterized by recording UV-VIS spectrum typically over 400 to 500 nm range on Beckman DU-20 Spectrophotometer. For the spectral scan, the nanoparticle solution was diluted with water (25 µl in 3 mL water) and transferred to a 1 cm path length plastic cuvette. De-ionized water was used as reference. The recording of the UV/VIS spectrum is a quick, convenient and easy way to establish the formation of silver nanoparticles. It takes advantage of strong absorption by silver nanoparticles (< 50 nm in size) in the visible range (390 to 500 nm). Strong absorption is the result of plasmon resonance band of nanometer size silver particles. Such spectral evidence is indirect evidence of silver nanoparticles.

Method A was used to investigate the effects of Tween 20 concentration, the molar ratio of silver nitrate to sodium saccharinate, silver nitrate concentration and TEMED concentration on nanoparticle formation. Tables 5 to 8 show the experimental details. The surfactant, sodium saccharinate, silver nitrate solution and TEMED volumes were in 10:10:10:1 ratio unless stated otherwise. See Figure 10 for measurements relating to Table 5. See Figure 11 for measurements relating to Table 6. See Figure 12 for measurements relating to Table 7. See Figure 13 for measurements relating to Table 8.

Table 5: Variation of Tween 20 Surfactant Concentration

Exp No.	Tween 20 (g/L)	$NaSac^+$ soln (M)	$AgNO_3$ soln (M)	TEMED (ml)	Precipitate/ debris?	Solution appearance

1	16.5	0.125	0.1	0.3	Yes	Dark brown, no Ag deposit
2	11.0	0.125	0.1	0.3	Yes	Dark brown, no Ag deposit
3	5.5	0.125	0.1	0.3	Yes	Dark brown, no silver deposit
4	0	0.125	0.1	0.3	Yes	Ash green
5	0	0.0625	0.05	0.3	Yes	Ash green
6	0	0.03125	0.025	0.3	Yes	Ash green

+ = Sodium saccharinate

Table 6: Variation of Sodium Saccharinate Concentration

Exp No.	Tween 20 (g/L)	$NaSac^+$ soln (M)	$AgNO_3$ soln (M)	TEMED (ml)	Precipitate /debris?	Solution appearance
1	16.5	0.125	0.1	0.3	Yes	Dark brown, no Ag deposit
2	16.5	0.110	0.1	0.3	Yes	Dark brown
3	16.5	0.105	0.1	0.3	Yes	Dark brown
4	16.5	0.102	0.1	0.3	Yes	Dark brown
5	16.5	0.100	0.1	0.3	Yes	Dark brown
6	16.5	0.075	0.1	0.3	Yes	Dark brown
7	16.5	0.050	0.1	0.3	Yes	Dark brown
8	16.5	0.025	0.1	0.3	Yes	Dark brown

Table 7: Variation of Silver Nitrate Concentration

Exp No.	Tween 20 (g/L)	$NaSac^+$ soln (M)	$AgNO_3$ soln (M)	TEMED (ml)	Precipitate /debris?	Solution appearance
1	16.5	0.1250	0.1	0.3	Yes	Dark brown, no Ag deposit
2	16.5	0.0625	0.05	0.3	Little debris	Brown/yellow, Ag deposit
3	16.5	0.03125	0.025	0.3	No	Brown/yellow

Table 8: Variation of TEMED Amount*

Exp No.	Tween 20 (g/L)	$NaSac^+$ soln (M)	$AgNO_3$ soln (M)	TEMED (ml)	Precipitate /debris?	Solution appearance
1	16.5	0.125	0.1	0.6	Yes	Dark brown (purple tint)
2	16.5	0.125	0.1	0.9	Yes	Dark brown (purple tint)
3	16.5	0.125	0.1	1.2	Little debris	Dark brown (purple tint)

* = The volume ratio was increased in favor of TEMED without changing volumes of other reactants

Effect of Tween 20 concentration

When the Tween 20 concentration was varied between ~ 5.5 gm/L and 16.5 gm/L little variation in the color and consistency of the nanoparticle solutions was observed. All showed characteristic yellow brown color. The white precipitate observed in the solutions was the undissolved silver saccharinate. No debris due to nanoparticle agglomerates, which normally would be black, was seen.

Figure 10 shows the normalized UV-VIS spectra of nanoparticle solutions with different amounts of Tween 20. The spectra of solutions without Tween 20 was not measured. All spectra are almost identical indicating that all three nanoparticle solutions are practically the same. The spectral wavelength maximum falls around 415 nm. A full width at half maximum (FWHM) ~ 90 value can be inferred (by extrapolating the curve between 350-400 nm maintaining symmetry) and is consistent with published literature. No agglomeration of nanoparticles was observed despite employing silver salt concentrations that were 10 to 100 times higher than used in published reports. This was unexpected because previous researchers have reported their inability to obtain stable nanoparticle solutions for silver concentration above 0.01M even after employing surfactants.

It is clear that stabilized silver nanoparticle solutions with a 0.1M silver concentration are achieved even with a low Tween 20 concentration of ~ 0.2% w/v. The data underscore the robustness of the preparation method. However, without Tween 20 in the solution, the nanoparticles agglomerated to form ash green colored precipitate. This was true regardless of the starting silver concentration. All solutions without Tween 20 failed to develop characteristic yellow brown coloration.

The Tween 20 concentration was also varied on the higher side i.e. 33 gm/L, 49.5 gm/L and 66 gm/L with matching increase in TEMED concentration. While we continued to see nanoparticle formation from the solution color and the observation of some debris that precipitated from the reaction mixture, the spectral signature of the solutions with higher Tween 20 remained essentially similar (data not shown) again verifying the process robustness. The data suggested that there was no advantage from the process point of view in raising surfactant content beyond the nominal value of 16.5 gm/L. However, higher concentrations of surfactant Tween 20 or other stabilizing agents can still b used without departing from the scope of the invention.

Effect of Sodium saccharinate concentration

The silver nitrate concentration was held at 0.1M and the sodium saccharinate concentration was varied to maintain ratios of saccharinate to nitrate between 0.025M and 1.25

to test the effect of modifying the saccharinate concentration (Table 6). Though, higher non-limiting ratios of saccharinate salt or salts of other anions can be used without departing from the scope of the invention. Ratios other than specified here may also be used. In all cases, whether the ratio was >1 or <1, yellow brown colored silver nanoparticles solutions were obtained with the debris primarily consisting of undissolved silver saccharinate. The spectra were practically the same (see Figure 11) indicating the nanoparticles sizes and distribution were with an average size of 5 -10 nm.

Effect of Silver nitrate concentration

Keeping all other conditions including the molar ratio of saccharinate to nitrate unchanged but varying the silver nitrate concentration did not affect silver nanoparticle spectra (Figure 12). The data once again indicated that the nanoparticle size and size distribution essentially remained unchanged. The appearance of the solution also stayed the same i.e. yellow brown with little or no debris (Table 7). These results gave the basis to use silver nitrate concentration to vary final silver nanoparticles count in the liquid composition depending on the product specification.

Effect of TEMED concentration

In the experiments above, the TEMED to silver nitrate solution volume ratio 1: 10. Here that ratio varied between 2:10 to 4:10 and looked for any changes in nanoparticle solutions formed (Table 8). Visually, the solutions remained similar but we also observed a purple tint on vial walls when we increased TEMED concentration.

The silver nanoparticles character (size and distribution) did not change as the spectra are identical (Figure 13).

Effect of order of reagent addition

In all experiments above, Method A was used where silver saccharinate was formed first. In Method B, silver nitrate was added last and in varying amounts. All resulting nanoparticles solutions showed little or no debris indicating no agglomeration. No undissolved saccharinate precipitate was seen. The test tube walls also had no metallic silver deposition indicating that the nanoparticles formed stayed in solution. Out of the 4 tests performed, the one where nitrate and saccharinate solution was in 3:4 ratio (0.75 ml in Fig 14) gave qualitatively the best solution.

Figure 14 shows spectra of four solutions prepared by reverse addition. In each case the wavelength maximum was 415 nm and the shape of the spectra over 400 to 500 nm range matched. For one solution, OD below 400 nm up to 350 nm was measured to see if there was

spectral symmetry around the maximum. The graph does indicate that the spectrum is symmetrical.

In comparison to known silver nanoparticle containing compositions , the nanoparticle compositions of the present invention comprise silver nanoparticles in concentrations almost 4 to 15 times or in some cases even higher based on the OD values as measured by UV-VIS spectrophotometer. This higher silver concentration gives added advantage to the compositions of the present invention in its ability to impart higher silver loadings on surfaces contacting the compositions,.

During the process parametric study, in a large number of the tests conducted there was the presence of precipitate or debris in the reaction vessel and occasionally on treated devices. However, this should not be construed as a limitation of the present invention. The precipitate present in the compositions is entirely due to the poorly soluble silver salt that is formed. By adjusting the starting concentration of soluble silver salt or by appropriate dilution, the amount of weakly soluble salt that may stay behind as precipitate can be reduced or eliminated.

Stability of silver nanoparticles solutions

Another important parameter from a process point of view is the stability of silver nanoparticles solutions as a function of time. Demonstrating at least a few weeks of stability is quite important. One indirect measure of stability would be no change in UV-VIS spectrum which can be easily monitored with time. In Figure 7 the UV/VIS spectra of saccharinate based aqueous silver nanoparticles composition made fresh and one of the same composition after 11 months period is presented. During this time, the sample vial was stored at ambient temperature (22C-25C). No change in spectra was seen between a freshly prepared solution and the stored one, even after nearly a year. This data support a finding that the silver nanoparticles solutions possess excellent room temperature stability. Similarly, though there is small nominal change in the spectra, there was good stability of a chloroform based non-aqueous silver nanoparticles composition at 4° C for over 3 months (Figure 15). The overall shape of the curve does not change much indicating the particles size and distribution does not change.

Compositional Ranges

The nanoparticle compositions may be derived from metal compounds formed in situ by anion exchange in an aqueous solution when a soluble metal salt such as silver nitrate and the sodium salt possessing the desired anion are mixed. For example, to form silver barbiturate, an exchange could occur between silver nitrate and sodium barbiturate. Silver compounds may be formed in situ or may be provided as final silver compounds. Silver compounds commercially

available as powders or crystals can substitute the in-situ formed silver compounds in the preparation of nanoparticle compositions of the present invention. In the practice of the present invention, silver compounds as a single compound or mixtures including, but not limited to, acesulfame, alkyl carbonates, acetylacetonates, acetates, ascorbates, barbiturates, benzoates, bitartrates, bis (2ethylhexyl) sulfosuccinate borates, bromides, carbonates, chlorides, citrates, folates, fumarates, gluconates, halides, hydantoins, substituted hydantoins, iodates, iodides, lactates, laurates, oxalates, oxides, palmitates, perborates, phenosulfonates, phosphates, propionates, saccharin and derivatives, salicylates, sorbates, stearates, succinates, sulfadiazines, sulfates, sulfides, sulfonates, and tartrates. Another feature of the method of preparation of the compositions of the present invention is that the soluble silver salt is converted to a less soluble silver salt in situ. In the formation of the less soluble silver saccharinate in the methods of preparation of the present invention, an excess of alkali metal alkaline earth metal saccharinate is maintained. The molar excess of the saccharinate ranges between ratios of 1 and 5, ratios between 1.05 and 2, and ratios between 1.1 and 1.5. The anion exchanging metal salts must possess cations higher in the electronegativity scale than silver. Non-limiting examples of available metal cations are sodium, potassium, calcium, and lithium. Non-limiting examples of soluble silver salts are silver nitrate, silver citrate, and silver acetate. Any soluble silver salt may be employed.

An important feature of the nanoparticle compositions of the present invention is that compositions spanning wide ranges of concentrations can be made without encountering compatibility or formulation problems. Silver content of the nanoparticle compositions can vary anywhere in the range of 0.0001% to 10%, 0.1% to 2%, and 0.1 to 5 %, . When preparing nanoparticle compositions with silver content such as > 5%, silver may precipitate out as flakes (agglomerated state) if a sufficient amount of surfactant or stabilizer is not maintained. The precipitate can be removed by filtration

The stabilizing agents are useful in maintaining the nanoparticles compositions of the present invention and can be a surfactant or a polymer. The surfactant can be of any type-anionic, cationic, nonionic, or amphoteric. A large variety of surfactants are commercially available. Non-limiting examples of stabilizers for use in the antimicrobial silver compositions are anionic, nonionic and amphoteric surfactants. Different classes of compounds are commercially available under each type of surfactants. Among polymers, polyacrylamide and derivatives (homo- and copolymers having acrylamide moiety, acrylamide with one or two substituents on the nitrogen atom), methacrylamide polymers and derivatives (homo- and

copolymers having methacrylamide moiety, methacrylamide with one or two substituents on the nitrogen atom), polyamides and derivatives, polyurethanes and derivatives, polyamines and derivatives can be used. Surfactants for use as stabilizing agents are nonionic known as Polysorbates or Tween NN where NN is an integer equal to 20, 40, 60 and 80.

The surfactant or stabilizer concentration in the compositions in relation to silver content may vary between the weight ratio of 0.1 and 500 but the total stabilizer concentration should not exceed 40% of the weight of the compositions. A ratio of values of surfactant concentrations of Polysorbate type generally lies below 5% w/v in the compositions. However, when using the polymeric stabilizers the values may also be higher than 5% w/v. Higher amount of stabilizer readily stabilizes silver compositions with higher amounts of silver loadings.

In most published studies on the preparation of compositions comprising silver nanoparticles a need for a reducing agent is recognized. Inorganic reducing agents have been employed but due to their strong reducing capacity, the formation of silver nanoparticles does not proceed in a controlled fashion thus yielding large size particles and often broad size distribution. Not all organic bases, when used as reducing agents, necessarily yield small and uniform size silver nanoparticles. Illustrative examples though not limiting in any way of reducing agents for use in the preparation of the antimicrobial silver compositions of the present invention are tertiary, secondary and primary amines; tertiary, secondary and primary diamines; homopolymers or copolymers having primary amine, secondary amine and tertiary amine moieties. Amine compounds may be aliphatic or aromatic. An aromatic amide such as diethyl toluamide popularly known as DEET also can be used. Useful reducing agents are tertiary amines or diamines, including triethanolamine and N,N,N',N' tetramethyl ethylene diamine (TEMED). Polymeric compounds having a TEMED moiety in the pendant chain or in the main chain also may be employed as the reducing agent. The amount of the reducing agent in the compositions again in relation to silver can vary between the weight ratios of 0.1 and 500, ratios between 2 and 50, and ratios between 4 and 20. The reducing agent can be added neat or in a diluted form. Both these variations are encompassed by the present invention.

Non-limiting examples of the solvent bases for the antimicrobial silver compositions are water or water based solutions where water is at least the major component. Other miscible solvents such as lower alcohols (C_6 or less), lower diols (C_6 or less), THF, DMSO, DMF etc. can be used either singly or as multi-component mixtures with water. Non-limiting examples of non-aqueous solvents or mixtures thereof are chloroform, methylene chloride, acetone, methyl

ethyl ketone, cyclohexane, ethyl acetate, diethyl ether, lower alcohols (C_4 or less), lower diols (C_4 or less), THF, DMSO and DMF. A variety of solvents that are HAPS free as defined under the clean air act of 1990 can be utilized in the preparation of non-aqueous silver compositions of the present invention.

Antimicrobial Medical and Non-Medical Devices

One embodiment of the present invention comprises medical devices that are rendered antimicrobial using methods comprising contacting the surfaces of the devices with the nanoparticles compositions. Medical devices, without limitation, include catheters (venous, urinary, Foley or pain management or variations thereof), stents, abdominal plugs, cotton gauzes, fibrous wound dressings (sheet and rope made of alginates, CMC or mixtures thereof, crosslinked or uncrosslinked cellulose), collagen or protein matrices, hemostatic materials, adhesive films, contact lenses, lens cases, bandages, sutures, hernia meshes, mesh based wound coverings, ostomy and other wound products, breast implants, hydrogels, creams, lotions, gels (water based or oil based), emulsions, liposomes, ointments, adhesives, porous inorganic supports such as titania and those described in US 4,906,466, the patent incorporated herein in its entirety by reference, chitosan or chitin powders, metal based orthopedic implants, metal screws and plates etc. Synthetic fabrics, those based on nylon or its blends with other fabric making materials (silk, rayon, wool, bamboo, polyester, acrylic, acetate) impregnated with silver nanoparticles are contemplated by the present invention. Devices, medical including dental and veterinary products and non-medical, made of silicone, polyurethanes, polyamides, acrylates, ceramics etc., and other thermoplastic materials used in medical device industry and impregnated with silver nanoparticles using liquid compositions of the present invention are encompassed by the present invention. Various coating compositions for different polymeric or metal surfaces that can be prepared from liquid compositions are also covered by the present invention. Such coating compositions can be hardened by solvent loss or cured by thermal or radiation exposure. Another aspect of the present invention are the blends of antimicrobial liquid compositions of the present invention and other antimicrobial agents such as glasses and zeolites similar to those disclosed in US 6,248,342 and US5,049,139 and incorporated in their entirety herein by their reference.

Antimicrobial medical and non-medical devices of the present invention can be made by treating the devices with antimicrobial silver compositions of the present invention by different methods. One disclosed method of the present invention comprises steps of making the said compositions in liquid form, contacting the said compositions and the devices surfaces for a

sufficient period of time to allow accumulation of nanoparticles and then rinsing the excess of said composition away and drying the device. A modification of the disclosed method may involve drying the surface of material first and then rinsing off the surface to remove excess. The method of contact may be dipping the device in the said compositions or spraying the compositions on the device or coating blends of polymer solution and said compositions. A variation of the disclosed method can be employed to deposit different loadings of silver on the surface of tubing. For example, initially, one level of silver loading can be applied over the entire length of the tubing. Then, if needed, a second application can be made over 2/3rd length of the tubing and finally only a 1/3rd portion of the tubing may be treated yielding a tubing with three levels of silver loadings. Using this approach any particular deposition pattern of silver loading can be achieved. A similar approach can also be implemented over a flat material creating different silver loadings pattern over the entire area. One embodiment of the present invention having three levels of silver loadings can be a bathroom product such as shower curtain. In such a product, the lower portion can be loaded with the highest level, the middle portion with intermediate level and the top portion with smallest level of silver. Such silver based curtain will prevent the mold and mildew formation on the curtain.

Yet another modification of the above disclosed method comprises steps of pre-treating the device surface with an agent that enhances the adhesion of silver nanoparticles to the surface or primes the surface to catalyze the silver nanoparticles formation by reduction of the silver salt amine complex that adsorbs on the surface. For example, g-aminopropyl triethoxysilane or similar type of adhesion improving agent, such as a polar compound, can be used. In another situation, the surface can be primed by treatment with an aqueous solution of tin chloride, rinsed with water, dried and subsequently treated with the aqueous silver nanoparticles composition, washed and dried to complete the silver deposition on the surface. In place of tin chloride, other agents such as gold, platinum, palladium, copper compounds can be used.

An important feature of the method of the present invention disclosed above is to deposit very small levels of silver loading uniformly on a surface. The surface may comprise a flat area, or belong to a sphere, cylinder (solid or hollow) and can possess nanometer sized features or micron sized features. The surface silver loading levels contemplated by the invention can be varied to meet the intended use, and may generally range from 0.1 ug/cm^2 to 100 ug/cm^2, 0.5 ug/cm^2 to 50 ug/cm^2, and 5 ug/cm^2 to 30 ug/cm^2.

A method of preparing antimicrobial medical devices such as hydrophilic foams, sheet dressings, fabrics, gauzes comprises of the following steps: immersing the dressing in

antimicrobial aqueous composition, draining the excess liquid or blotting it away, then re-immersing in a second non-aqueous liquid such as ethanol, isopropanol or THF for a period effective enough to destabilize the silver nanoparticles, thereby depositing them permanently on the substrate, blotting away excess liquids and finally drying the substrate device. A modification of the method may comprise adding the antimicrobial silver nanoparticle composition to the starting mixture of ingredients to prepare a device (e.g. a polyurethane based foam).

A method may comprise forming a liquid layer or film of the pre-mixed composition (composition that is not yet subject to a temperature increase) on the desired surface and then using known means to rapidly cause a temperature increase of the liquid film or layer to initiate silver nanoparticle formation in the vicinity of the surface to which the nanoparticles irreversibly adhere to yield an antimicrobial surface. The means to rapidly increase temperature may include acoustic radiation, microwave radiation and IR radiation or other electromagnetic radiation. Thermal energy can also be provided by way of an oven-like environment.

Yet another method disclosed for rendering medical devices antimicrobial particularly those that can withstand higher temperatures (without losing dimensional integrity) comprise the steps of preparing the pre-mix composition, heating the medical device to uniform temperature, spraying or dipping the device with the pre-mix composition to initiate rapid reduction of the silver compound in the liquid film adhering the devices surface to silver nanoparticles that irreversibly attach. If the device is dipped then it can be removed from the bath to dry the liquid film and the devices surfaces rinsed cleaned with water or other solvents. If the warmed device is sprayed then the liquid will be evaporated off from its surfaces. The surfaces can be rinsed with water or similar solvents. The rinse solution may be plain water or may comprise other additives such as surfactants, acids or complexing agents.

Modifications of the methods of the present invention for rendering certain hydrophobic polymers antimicrobial may be required. For example, silicone polymer surfaces may not readily becoming antimicrobial by immersion in aqueous silver compositions. One disclosed embodiment comprises a method comprising the steps of immersing the silicone polymer in a swelling solvent (that is also miscible with water) to effectively fill the pores with swelling solvent, transferring the swollen silicone polymer substrate quickly and immersing it in the aqueous silver composition of the present invention for a specified period to cause the exchange of solvent within the pores. As a result, the silver nanoparticles from the aqueous composition are drawn into the pores thus rendering the silicone polymer surface antimicrobial.

Medical devices or non-medical devices of the present invention can also be treated with non-aqueous silver compositions. Often the devices comprising alginates or CMC either as fibers or foam fibers are not suitable for treatment using aqueous compositions as they are unusable after coming in contact with water rich composition. Instead such devices can be conveniently treated with non-aqueous silver compositions by dipping method or spraying the compositions on the substrates. After removal of solvent that occurs by evaporation under normal conditions or by vacuum, the surfaces of the devices are impregnated with silver nanoparticles and becoming antimicrobial. Non-aqueous compositions can also be used to treat medical devices made from other polymers so long as the non-aqueous solvent is a non-solvent for that polymer or does not diffuse into the device and cause swelling. Non-aqueous silver nanoparticle compositions can also be used in situations where swelling is not detrimental. For instance, PTFE films can be rendered antimicrobial by briefly dipping them in a chloroform solution of silver nanoparticles. Such solution also can be sprayed to yield pale yellow colored PTFE.

Yet another distinguishing feature of the present invention is a method of forming silver nanoparticles in situ on the surface of a medical device. For instance, one disclosed embodiment comprises a method of yielding an antimicrobial surface comprising the steps of providing a surface coating comprising finely dispersed particles of the silver compound and treating the coated surfaces with a reducing agent for a specified period or until all of the silver compound is reduced to silver nanoparticles predominantly monodisperse in size. An example of a silver compound that can be used in such a method is silver saccharinate. A reducing agent is TEMED and can be used to carry out the reduction at room temperature. Though not limiting, room temperature is preferable for this method though higher temperatures can be employed without departing from the present invention. The silver nanoparticle compositions can be formed in situ in a polymeric coating or in porous matrices such as ceramics, clay, zeolites, alumina, silica, silicates with finely divided silver compounds and saccharinate in particular by reduction with TEMED or similarly listed amine compounds.

Utilizing the methods of preparation of the present invention rendering a device surface antimicrobial can yield different amounts of silver loading depending upon the treatment conditions. However, a commercial process requires that the silver loading meet the specifications. In the instances where the silver loading may exceed the upper specification limit, the product batches may be rejected incurring significant costs. In such instances, it is desirable that the product batch be re-treated to bring the silver loading within the specification.

One disclosed method of the present invention to re-treat the device surface impregnated with excess silver nanoparticles comprises the steps of,

(a) preparing a solution of 0.5% to 15% nitric acid,

(b) treating the device surface with the said nitric acid solution for a specified period by immersing the surface in the solution and,

(c) thoroughly rinsing the device surface with deionized water and drying.

This method can remove the impregnated silver selectively in small portions and also can be utilized to completely strip the silver off the device surface or to clean production equipment. This method also can be used to strip silver off of a treated surface to create patterned surfaces bearing silver nanoparticles.

Another embodiment of the present invention discloses a method for altering the amber or yellow brown color of the antimicrobial medical and non-medical devices deposited with silver to improve their aesthetic appeal. Yet another feature of the present inventive method is that it can cause uniform color loss of amber color of the silver nanoparticles bearing surfaces without loss of silver. Even very hard to reach surfaces typical of some pre-formed micron sized objects can be readily treated as the peroxide solution can readily penetrate and wet most surfaces. The inventive method comprises following steps of,

(i) preparing an aqueous solution of hydrogen peroxide in appropriate concentration,

(ii) treating the amber colored surfaces comprising silver nanoparticles for a specific period,

(iii) rinsing off the treating solution thoroughly with deionized water and drying the surfaces.

The hydrogen peroxide concentration in the treating solution can be varied from as low as 3% to 30% by weight. The time period of contact of surfaces with the treating solution will be dictated by the peroxide concentration in solution. For instance, the rate of color loss of amber color is slower at low peroxide concentration and vice a versa. The duration of contact also depends upon the product specification. If a product needs to be distinguishable as a silver containing product from non-silver containing product one may want to terminate the peroxide treatment to leave behind a faint yellow tint to the surface. In addition to water as the solvent for peroxide solution, small quantities of solvents miscible with water (but those non-reactive to peroxide) may be added.

One may provide hydrogen peroxide as vapors with or without an inert carrier such as nitrogen to cause contact with the surfaces to be treated without departing from the scope of the

invention. The use of temperatures above and below room temperature in the peroxide treatment of silver nanoparticles comprising surfaces are also encompassed by the present invention. Other methods such as the use of ultrasonic energy to increase the color loss by peroxide treatment also can be employed. Patterning surfaces bearing silver nanoparticles by the hydrogen peroxide vapors or aqueous solutions by appropriate masking is covered by the present invention.

Nanoparticles, such as silver nanoparticles, can be used to create a nanoparticle coated foam or porous matrix that can be simply added to non-potable water to disinfect it. Such a product may be more appealing to campers over current iodine based products as there water with trace amount of silver has no taste. In the construction industry, for the control of mold and mildew in homes the wooden structures during construction may be sprayed with antimicrobial silver compositions of the present invention.

The present invention also contemplates antimicrobial radioactive silver (^{110m} Ag) compositions and their methods of preparation. In the use of these compositions, the antimicrobial property can be a concomitant property. These compositions can be used to prepare radioactive tracers comprising ^{110m}Ag nanoparticles. One potential use of these compositions is to prepare labels with small amount of ^{110m}Ag nanoparticles adhering to them. Such labels can be readily prepared by spitting tiny drops of the solution on the label surfaces by inkjet printing methods. Such labels can then be used where a product has shelf life equal to the half life of ^{110m}Ag. Because the amount of radioactive ^{110m}Ag is so small there is practically no risk of harm to consumer or to the product. They also may be used as tracers in security applications e.g. in authentication.

One embodiment comprises a method of preparation of antimicrobial radioactive ^{110m} Ag nanoparticles composition comprising the steps of,

(i) preparing a stabilizer solution,

(ii) successively adding to it the sodium or suitable metal saccharinate solution, ^{110m} Ag nitrate solution, reducing agent solution and,

(iii) causing a temperature increase to initiate reduction of in-situ formed weakly soluble silver saccharinate to form silver nanoparticles.

Optionally the temperature increase may be for a brief period or may be maintained for a specified period.

Mechanism of Silver Release from Solid Surfaces

An aspect of the nanoparticle compositions is their ability to efficiently deposit metal on surfaces in the form of nanoparticles that adhere to surfaces strongly. Not only does the deposition of nanoparticles take place, simple handling will not dislodge the nanoparticles from the surface. They even cannot be readily removed by ultrasonication suggesting practically irreversible binding of the nanoparticle to the surface. However, the nanoparticles can be disrupted or dissolve away if chemically treated.

While the presence of elemental silver on the surface would generally make that surface at least bacteriostatic, it would not necessarily make it bactericidal. Even if it did, it would be extremely difficult to sustain such an action. Increasing silver loading may increase sustained release but it also increases the risk of cytoxicity in end use. The nanoparticles or nanoparticle compositions of the present invention possess the ability to impart antimicrobial characteristic to surfaces that can sustain the activity for long periods without being cytotoxic to mammalian cells. Figure 16 shows the amount of silver released (as ions) each day from a nylon surface treated with said antimicrobial silver composition. There is sustained prolonged antimicrobial activity because the only change taking place on the surface after treatment with the compositions is the impregnation by silver nanoparticles. As the activity is due to silver ions, it is clear that the only source of silver ions is the silver nanoparticles. The results indicate that an effective amount of silver ions is released on a continuous basis over long periods. The results were also confirmed by a test carried out using nylon tubing impregnated with radioactive silver nanoparticles. The release characteristics of radioactive silver (Figure 16) at similar silver loading are comparable to those observed earlier.

Because it is well established that it is the silver ions (Ag^{+}) that bring about the antimicrobial action not Ag^{0}, it is believed that the source of antimicrobial silver ions are the silver nanoparticles residing on the surface. The present results show, sustained release of ionic silver from nanoparticles made by the methods taught herein. Theoretical estimates show that at the observed rate of egress of silver from the surface, it would take over 150 days to completely deplete the silver, which is extraordinary.

Other Applications

The antimicrobial silver compositions of the present invention can also be the starting material for producing dry silver nanopowders suitable for material science and metallurgical applications. Such compositions, aqueous or non-aqueous could be atomized in high temperature environment to produce dry silver nanopowder. The compositions of the present invention can be produced on a large scale and, because they are prepared from relatively

inexpensive chemicals, a commercial process could be quite feasible and could compete with other dry processes for silver nanopowder. Another advantage of the compositions of the present invention in producing dry silver nanopowders is that the nanoparticles average size of ~ 10 nm is small and the size distribution is relatively tight- two factors that could offer competitive edge over silver nanopowders with broad size distribution produced by dry processes.

Other applications for silver nanoparticles comprising compositions of the present invention are in the catalysis of oxidation of olefins, separation of olefinic compounds, as polishing slurries, dissipation of static charge from surfaces, increasing thermal conductivity of liquids, increasing electrical conductivity, in the preparation of radio frequency or similar radiation shields, in analytical chemistry for surface enhanced Raman spectroscopy.

In one aspect, the present invention provides a method of depositing silver nanoparticles on elastomeric articles (e.g., those made of silicone) and optionally, rendering the elastomer conductive, and the silver nanoparticles coated elastomeric articles produced therewith. The term "conductive," as used herein, refers to a conductivity of the order of about 0.1 Siemens/m or more. A conductive article includes a semi-conducting article and an article with metal-like conductivity.

Among noble metals, silver is quite versatile as its antimicrobial properties finding widespread uses in biological and medical applications and its high electrical conductivity and thermal conductivity finds applications in electrical, electronics, and thermal transfer fields.

One application utilizing high electrical conductivity of silver is in making conductive elastomers. The term "elastomer" is meant to encompass materials that can withstand strains of 1% to as high as 1000%. Such elastomers (e.g., elastomeric sheets or gaskets) may contain up to about 60% silver in the form of fine powder to yield high conductivities. These elastomers typically can maintain their conductivities even after being stretched by 300%. In sheet forms, these elastomers may be applied to a surface to absorb radio frequencies, for example, thus making the surface invisible to radar detection, thereby making it potentially useful for military applications. However, with 60% of the weight of the sheet elastomers being silver, it will add considerable weight to an aircraft if the sheet elastomers are to be used. Thus there is need for silver based conductive elastomers that can provide radar invisibility and yet not add much weight to the base weight of the aircraft. In addition, the conductive elastomer may also be stretched significantly, for example, 300%, without loss of conductivity.

In one embodiment, the present invention provides methods for the deposition of silver nanoparticles on to an elastomeric article, comprising contacting an elastomeric article with a silver composition under conditions suitable for reducing silver ions to silver nanoparticles, thereby providing a silver nanoparticles coating to at least one surface or a portion of a surface of the elastomeric article. The silver composition may comprise a silver salt, a solvent, a reducing agent, and a stabilizing agent. In another embodiment, the silver composition may comprise silver nanoparticles produced in accordance with methods of the present invention. For example, a silicone-based elastomeric article may be immersed in a silver nanoparticle solution under conditions suitable for reducing silver ions to silver nanoparticles and for depositing the silver nanoparticles to the article. Upon completion of the silver deposition step, the article may be rinsed thoroughly with deionized water, sonicated to dislodge loosely adhering silver nanoparticles, and dried in an oven to eliminate moisture. The deposition process may be repeated to obtain a conductive elastomeric article with desired silver loading, i.e., the desired amount of silver nanoparticles present. The conductive elastomeric article of the present invention may also be treated with compositions, such as, Tollens reagent or its variants, to change, modify, or improve its physical or functional properties.

The present invention in a general sense comprises methods of preparing conductive elastomeric articles. The present invention also covers the compositions comprising silver that are employed in said methods, articles prepared using the said methods and the methods of using the said articles in various applications.

One embodiment of the methods of preparing flexible conductive elastomers comprises of (i) cleaning the virgin elastomer surface, (ii) depositing silver nanoparticles on the flexible elastomer surfaces, (iii) rinsing the treated surface and, (iv) drying to get rid of moisture or solvent. Optionally after obtaining a dried silver deposited surface, it may be annealed to increase the strength of the silver coating deposited. To improve the adhesion of silver to the surface, optionally a wet or dry chemical treatment step after cleaning step above but before the silver deposition step may be included.

The purpose of cleaning virgin surface is to ensure a baseline clean surface. However, this step can be omitted without departing from the scope of the invention. Cleaning step can be achieved by the use of known solvents such as high purity de-ionized water, isopropanol, ethanol, glycol, acetone, toluene, naphtha fractions, fluorinated solvents, acetate based solvents and mixtures thereof. Aqueous solutions may optionally contain surfactants, soaps, acids and detergents or be blends with organic solvents. It is understood that cleaning solvents or solutions

are selected so that they don't damage the elastomers by swelling or cracking the surfaces. The cleaning step may involve only a single rinse or multiple rinses or single rinses using different types of solvents or solutions. One can rinse with an organic solvent (that is miscible with the follow on solvent) followed by a rinse with another appropriate solvent (either aqueous or non-aqueous). A variety of solvent combinations are possible with the overall objective of achieving a clean surface. The type of cleaning solvents and solvents listed above are presented for illustration and by no means should be construed as limiting.

In addition to the wet cleaning step disclosed above, dry processes may also be employed to complete the task. For example, plasma based cleaning processes (oxygen or mixtures of gases) may be used to clean surface. An additional benefit of plasma cleaning is to introduce polar groups on the surface especially on hydrophobic polymer based elastomers e.g. silicone. The plasma treatment may actually perform two functions; it may clean the surface and also increase surface polarity that would improve the adhesion of nanosilver to the surface. Alternately, a wet chemical treatment with appropriate solutions of □-aminopropyltriethoxysilane or with sulfur compounds such as thiogylcerol or dodecanethiol may be carried out to improve adhesion of silver nanoparticles to the flexible surfaces. The use of binding layers known to those ordinarily skilled in the art for improving adhesion is also contemplated by the present invention.

The deposition of silver nanoparticles on elastomeric surfaces is carried out by immersing the surfaces in a silver containing solution. The silver containing solution may be aqueous or non-aqueous. In one embodiment, the silver containing solution is made and then elastomeric samples are immersed in and treated for a pre-determined temperature and time. In another variation, the elastomeric samples are pre-arranged in a bath or a container and then the silver containing solution is poured over the samples and maintained at desired temperature for a given period. The surfaces to be coated may be flat or be vertical - both configurations are encompassed by the present invention. In another embodiment it may be desirable to deposit silver only one side of a slab or sheet. In yet another embodiment it may be desirable to deposit silver only in unmasked area to form traces. In yet another embodiment, the silver can be deposited to conductive levels on flexible or non-flexible substrates having channels and selectively remove silver from non-channel area to form conductive channels. All such variations are encompassed by the present invention.

The silver deposition step may be carried out at room temperature or optionally below or above room temperature. Different levels of silver coating on flexible surfaces can be achieved

by the methods of the present invention. By varying the starting concentration of the silver in the treating composition or alternately at a given concentration carrying out the treatment at a higher temperature or for a longer period one can vary the level of silver loading in the deposited layer. One can treat the elastomeric substrate multiple times to increase silver loading even further. But generally less than five silver treatments are used. There are several embodiments on how the silver treatments may be carried out. For example, one may choose to apply silver coating by using silver nanoparticles compositions of the present invention in only one step or multiple successive steps.

In another embodiment, one may employ one treatment with silver nanoparticles composition and a second silver treatment such as with Tollen's reagents or its variants. In yet another embodiment, one may employ one or more silver treatments with silver nanoparticles compositions and then one silver treatment with Tollen's reagent or similar composition. It is understood by those skilled in the art that optionally one may include a sensitizing solution treatment before treatment with Tollen's reagent. Thus it will apparent to those skilled in the art that the methods of present invention allow for excellent flexibility in loading from small amounts of silver (for semi-conducting surfaces) to very high levels of silver to yield metal-like conducting elastomers. Though the values of conductivity for conductive elastomers of the present invention may be 0.1 S/m or more, one can prepare conducting elastomers with values < 0.1 S/m without departing from the scope of the invention. Alternately, the conductive elastomers may be characterized by the amount of metal deposited or coated on the surface. It is logical to correlate less or more amounts of silver per unit area with lower or greater levels of electrical conductivity. A range for the amount of metal in the electrical conductive layer of conductive elastomers of the present invention may be 0.03 mg/cm^2 - 50 mg/cm^2, 0.1 - 20 mg/cm^2, or 0.5 - 5 mg/cm^2.

The compositions used for depositing silver on flexible surfaces are not very different than those used for depositing silver nanoparticles on hard surfaces. A large number of compositions for preparing silver nanoparticles are disclosed in PCT/US2005/027261 patent application which is incorporated in its entirety by reference. Any of the compositions disclosed in that application may be used in the deposition of silver. In one composition, equal volumes of solutions of Tween 20, silver nitrate and sodium saccharinate were mixed under stirring followed by 1/10th the volume of Tween 20 solution as TEMED and used to treat flexible silicone substrate in the form of 3"x 1" strips 0.1" thick. It may be apparent to those ordinarily skilled in the art that during multiple treatments to achieve higher loadings of silver, one may

use a composition based on saccharinate as anion in the first treatment and an acetate based composition in subsequent treatments either once or more than once. Compositions based on the use of mixture of anions are also contemplated for use in the silver deposition step. Even the well known Tollen's reagent or its variants may be employed after the first tier treatment is by silver nanoparticles solution. In one embodiment of the present invention, after multiple treatments with silver containing composition, but before silver deposition with Tollen's reagent, the surfaces were treated with tin chloride solution. The tin chloride is used to "seed" the surface to accelerate silver deposition during treatment with Tollen's reagents. Those skilled in the art will recognize that salts of other noble metals such as palladium, copper, etc., may also be used in place of tin.

Compositions comprising TEMED and triethanolamine are used but compositions comprising any suitable initiators listed in the co-pending application no. PCT/US2005/027261 may be used for depositing silver on elastomeric substrates. Similarly, a variety of surfactants may be used in preparing compositions for depositing nanoparticles, such as polysorbates.

After the completion of the silver deposition, the surfaces are rinsed to remove excess silver solution and to wash loose silver particles. For rinsing, traditional rinsing methods may be employed. Silver coated parts may be sprayed with a rinse solvent which may be water. Parts may be rinsed by simply raising them up and down in the bath. De-ionized water may be used but water from municipal sources also may be employed to reduce costs. In such cases, the final rinse may be of de-ionized water. Additional rinsing with water miscible alcohol also may be carried out to dehydrate and dry the surfaces. High energy water jets or sonication bath also may be used to additionally remove any residual loose particles.

Drying of silver coated surfaces may be carried out by letting them dry under ambient conditions or by blowing hot air over the parts. The use of IR lamps or acoustic energy may be employed if the coated surface areas are relatively small.

Optionally, an annealing step may be carried out to fuse the metal nanoparticles coating to increase its strength. In the case of the annealing step the underlying substrate is not damaged by it. The annealing step may be carried out to provide thermal energy to the silver coating to cause its temperature to be at least between 100oC and the melting point of the metal by any known means. The precise temperature needed to effect proper annealing is dictated by the thickness of the silver layer to be annealed, the type of substrate used, the thickness of the article etc.

For instance, annealing may be carried out by exposing the silver coated surfaces to an open flame. An open flame that may be controllable may be generated using propane, butane, acetylene or similar gases. During annealing by flame, it is important that the surfaces may be kept moving so as not to cause hot spots. The silver coated substrates may be held in fixtures that would rapidly move the coated surfaces over the open flame. The fixtures are sufficiently flexible to allow for annealing of all surfaces with minimum difficulty.

Alternately, annealing may be attempted by placing the silver coated substrates in ovens or furnaces maintained at desired annealing temperature. In another embodiment the parts to be annealed may be placed on a conveyor belt that travels through a controlled temperature environment. In yet another modification, substrates may be held in a waffle iron type device to anneal surfaces from both sides simultaneously. In yet another modification, annealing may be carried out by exposing the substrates with silver nanoparticles with steam. The steam may be at low pressure or high pressure and may be dry or wet. Alternately, the silver coated substrates may be squeezed between hot rollers to bring about annealing of the silver coating. The use of electromagnetic radiations such as IR, high energy e-beam, x-ray, nuclear radiation, lasers etc in annealing step is also contemplated by the present invention. For examples, a high power laser beam can be traversed across a silver coated elastomeric substrate to create a known pattern of electrically conductive traces.

A variety of elastomeric substrates can be deposited by silver using the methods of the present invention. A select few but non-limiting examples include silicone, polyurethane, synthetic and natural rubber. For that matter, the underlying polymer, which may be a synthetic or natural polymer and may exhibit ability to withstand low or high strain are encompassed by the present invention. Flexible substrate may comprise all known synthetic and natural film forming polymers. Non-limiting examples of polymers include polyimides, polyamides, polyacetals, polysulfones, PBTs, PBO's, ethylene and propylene based polymers, acetate polymers, polyacrylates, polycarbonate, PET's, PEN's or blends thereof or co-polymeric derivatives. Though the substrates comprising the listed polymers may be flexible they may exhibit only low levels of reversible strain. Still these substrates are encompassed by the present invention.

Various embodiments of conducting elastomeric articles can be made by the methods of the present invention. In its simplest and perhaps the most useful form, elastomeric sheets or strips can be treated to deposit silver coating by inventive method or its variants disclosed above. An embodiment in the form a flexible conductive strip having multiple layers of silver or

any of the other metals mentioned can be made as follows. A strip of flexible substrate e.g. silicone is treated to deposit silver coating on both sides with larger surfaces and optionally annealed to obtain conductive silver coating. The strip is then treated with a solution of γ-aminopropyltriethoxysilane or a mixture comprising it to deposit a thin layer of the silane. After curing the silane layer, a viscous coating of silicone pre-polymer such as Sylgard 184 is applied on both sides of the strip to obtain a flexible conductive elastomeric article having two silver layers. In a variation of this embodiment, one can coat silver on both sides as before. Thereafter, one can use a silver dissolving solution e.g. concentrated nitric acid to remove coating one side of the strip. A layer of another metal then can be deposited on the stripped surface. Optionally, after annealing, a conductive elastomeric strip with metal layers of different kind is obtained. Again by applying a silicone coating on top of the metallic layer with or without a binder layer such as γ-aminopropyltriethoxysilane, a sandwich elatomeric article have two different metal layers is obtained. Following the method described, one can also make a conductive elastomer with more than two metallic layers.

Measurements for Conductivity

To assess if the surfaces of the elastomeric substrates after silver or metal deposition have enough conductivity, measurements using a simple multi-meter were carried out. However, there are more sophisticated techniques such as 4 point probe method that are commonly practiced in semiconductor industry for measuring conductivity values with which those ordinarily skilled in the art are familiar. The unit for reporting conductivity is siemens/mm or siemens/cm which is reciprocal of electrical resistivity reported in ohm-cm or ohm-m. It is straightforward to convert to one set of units from another set and vice versa. Other common unit for reporting conductive coatings is ohms/square which is related to bulk resistivity by the following equation.

$$R = r \cdot L/(t \cdot W)$$

Where, R is the reistance of the thin film coating with length L and width W, r is the bulk resistivity in ohm-meter or ohm-cm and, t is the thickness. For a square resistor, L=W and therefore R = r/t. Thus if thickness of the coating is known, the bulk resistivity r can be determined and hence conductivity can be calculated.

In the case of the examples disclosed in the application, the resistance values were measured using a multi-meter and reported. It is well understood in the art that when a measurable resistance reading is observed by the multi-meter it is a good indicator that an electrical continuity is established via the metal coating on the elastomeric substrate. For various

prototypes disclosed in the application, the electrical continuity remained established even after application of high axial, bending or torsional strains as indicated by measurable readings of the resistances by multi-meter.

The methods of preparing elastomeric conductive articles comprising silver or other metals disclosed herein yield articles that show conductivity under high strain conditions. Without being bound to any particular theory, it is theorized that the nanoparticles are deposited on the elastomeric substrate and fill the surface voids densely, yielding an electrically conducting layer. Further annealing of this layer increases its strength modulus that allows this layer to stretch readily even under high strain without failure. As a result electrical continuity is maintained.

The present invention further provides methods of using conductive silver elastomeric articles, such as, without limitation, to provide fireproof capability, to reduce electromagnetic interference, to shield devices and circuits against electrostatic discharging, and to impart radar invisibility to military aircraft or other vehicles. To provide fireproof capability, it is not essential that the elastomers have to be electrically conductive.

In one aspect, the present invention provides methods and compositions comprising for forming anti-fouling coatings to an article, and the anti-fouling coatings produced therewith. Materials that are immersed for long periods of time in fresh or marine water are commonly fouled by the growth of microscopic and macroscopic organisms. The accumulation of these organisms is unsightly and in many instances interferes with function. The natural process of accumulated growth is often referred to as fouling of the surface. There are a number of agents that may be applied to the surfaces to inhibit this growth. These agents are known in the art as anti-fouling agents. While these agents are highly effective, they have a fundamental limitation in that they contain extremely toxic agents that often leech from the surface of the article and accumulate in the local environment. Tin, copper and zinc are examples of the agents that cause such problems when used to kill local biota. Silver has been proven to be well tolerated by the biota in the surrounding area yet be an effective way of eliminating fouling of treated surfaces. In one embodiment, the present invention provide a surface functionalization process, where an anti-fouling coating to an article may be formed in accordance with a method comprising contacting the article with a silver composition under conditions suitable for reducing silver ions to silver nanoparticles, thereby providing an anti-fouling coating to at least one surface of the article, wherein the silver composition comprises a silver salt, a solvent, a reducing agent, and a stabilizing agent. For example, a surface functionalization process is taught herein for the

formation of a silver salt of saccharinate in water containing a stabilizer agent (e.g., Tween) and a reducing agent (e.g., TEMED) which upon mild heating forms silver nanoparticles. Different types of surfaces can be treated using the surface functionalization process and such treatments and surfaces are contemplated by the methods of the present invention, including steel, stainless steel, glass, titanium, copper, gold, and a variety of polymers, such as, polypropylene, polycarbonate, polyurethane, polyvinyl chloride, polystyrene, polysulfone, and a number of silicones, including HTV and RTV. In some embodiments, the silver nanoparticles so formed are bound to the materials very tightly, some are bound tightly so that they can not be dislodged by even sonication.

The fouling of surfaces which are exposed to fresh and marine water in nature is thought to be due to the formation of a biofilm, the articles with the silver nanoparticle coatings of the present invention were tested for their resistance to biofilm formation. The experimental data indicates that such articles coated with nanoparticles resist biofilm formation. For example, the surfaces of stainless steel and plastics (e.g., polycarbonate and polypropylene) may be contacted with silver or other metal nanoparticles using methods in accordance with embodiments of the present invention. Such materials may be widely used for food processing or storage equipment, which is prone to the formation of biofilms during use. The surfaces treated in accordance with the present invention prevent or reduce biofilm formation and thus minimize or reduce the likelihood of transmission of microorganisms which may cause food spoilage and disease. Any article or surface that contacts a fluid and which could have a biofilm attach or grow can be treated by the methods and compositions taught herein. Examples of such articles or surfaces include, but are not limited to, food storage and preparation devices, laboratory equipment, marine or water vehicles, hulls, propellers, anchors, ballast tanks, motors, pilings, liquid filtering equipment, tubing, ropes, chains, fish tanks, liquid containers, water bowls, cooling towers, water tanks, canteens, fuel tanks, and storage bins.

Antimicrobial surface coatings, such as, the antimicrobial silver coating of the present invention, may prevent transmission of disease between persons and/or animals. Surfaces that are touched by humans or animals may be treated by the methods and compositions taught herein and thus are made resistant to transmission of microbes. This lessens the risk of transmission of microbes of the environment. For example, the surface of a golf ball is very often cleaned by golf players either by licking or by moistening with saliva. Therefore ample opportunity exists for the transfer of organisms from the surface of golf balls to the buccal cavity of the players. Many of the organisms that may reside on the surface of a golf ball may post

severe health risk to people. In one embodiment, the present invention provide a method for forming an antimicrobial coating on a golf ball, comprising contacting a golf ball with a silver composition under conditions suitable for reducing silver ions to silver nanoparticles, thereby providing an antimicrobial coating to the golf ball, wherein the silver composition comprises a silver salt, a solvent, a reducing agent, and a stabilizing agent. Any common article having a surface that may contact a human or animal can be treated using the methods taught herein to provide an antimicrobial coating to the article.

The present invention further provides a method for making ultra-smooth surfaces for applications in a wide range of fields, including, without limitation, electronics and medicine. In one embodiment, the method comprises contacting an article with the silver composition taught herein under conditions suitable for reducing silver ions to silver nanoparticles and orderly binding the silver nanoparticles to at least one surface of the article, wherein the silver composition comprises a silver salt, a solvent, a reducing agent, and a stabilizing agent. Under such conditions, the silver nanoparticles formed will attach to the surface. Electron microscope images of the location of the particles show that they may be evenly distributed on the surface when the surface is very smooth. When the surface is irregular or rough, such as, containing pitting, grooving, depressions, and/or extrusions, the deposition of particles is initially in the lower parts of such depressions. As more particles become deposited there is a tendency for the depressions (e.g., grooves and pits) to be filled first. The remainder of the surface is then coated by a more even distribution of the particles. This process may form a very smooth surface coating, i.e., a new surface on top of the coated surface. In the case of the silver nanoparticles the effect is the formation of an ultra-smooth and highly reflective surface.

In more than one embodiment of the present invention, silver nanoparticles with a diameter ranging from about 0.5 to about 100 nanometers may be attached to surfaces. The union with surface may be independent among the particles so that the particles may be relatively independent from their adjacent particles. Such an application of silver nanoparticles may produce a beneficial effect for the treatment of flexible materials, such as, without limitation, balloons, and synthetic or natural polymers. Surfaces so treated with silver nanoparticles, even to a sufficient density to become reflective and conductive, may be flexed, stretched, and/or relaxed multiple times without causing the applied silver nanoparticles to fall or flake off of the surface. Such characteristics make the methods of the present invention useful for the production of, for example, flexible mirrors and stretchable elastic conductive polymers.

Microbiological Testing

The antimicrobial activity of device prototypes made with antimicrobial silver compositions was verified by standard zone of inhibition microbiology assay using *Staphyloccocus aureus* ATCC 6538 bacteria. Disks of ~ 5-7 mm size were cut from samples and placed on a Mueller Hinton Agar (MHA) plates that were inoculated with bacteria and incubated overnight at 37C. Disk from which silver ions were released showed a clear zone around them. Untreated samples and Silvasorb served as negative and positive control respectively. The results from zone of inhibition assays are presented in Tables 9 and 10. Because the device prototypes comprise silver nanoparticles and not silver salts, ZOI assay may not be the most suitable screening assay for antimicrobial activity. Therefore, often we employed a bacterial challenge test to evaluate microbiocidal activity and sustained release characteristics. In an 8 hour bacterial challenge assay, catheter sample pieces were immersed in culture medium in tubes and inoculated with bacteria. The tubes were incubated at 37C for 8 hours after which aliquots of culture medium were diluted and spread on MHA plates and the numbers of bacterial colonies grown after 24 hour5s were counted to determine the kill rate.

Liquid compositions with slightly different compositions (see descriptive examples) were prepared quite readily and used to impregnate variety of substrates with silver nanoparticles including cotton gauze, nylon fiber and contact lenses and hydrogel sheet. All prototypes including amorphous talc powder showed zones of inhibition and sustained release antimicrobial

Table 9: ZOI Assay using *Staphylococcus Aureus*
(Zone of inhibition+disk dia/disk dia)

Example	Substrate	ZOI data	Example	Substrate	ZOI data
A1	Cotton gauze	9.5/7.0	A11	Cotton gauze	4.0/1.0
A2	Cotton gauze	9.0/6.5	A12	Cotton gauze	3.0/1.0
A3	Contact lens	8.0/6.5	A13	Contact lens	11.0/7.0
A4	Si catheter	4.5/4.0	A15	Nylon catheter	3.0/1.0
A5	Hydrogel	16.0/8.5	A16	Nylon catheter	7.0/1.0
A6	Contact lens	9.0/6.5	B9	Lubricating jelly	6.0/5.0
B1	Hyd* polymer	8.5/6.0	B10	Alginate beads	7.0/3.0
B2	Hyd. Poly w/ copperCu	10.0/5.0	A18	Breast implant membrane	8.0/6.0
B4	Talc powder	7.5/7.0	A7	Nylon fiber	4.0/1.0
A9	Catheter w/ hyd. Poly.coating	6.0/4.5	B15	Polypropylene woven fabric	9.0/7.0
A10	Contact lens	10.0/6.0			

*Hydrophillic

activity against *Staphylococcus aureus* (see Table 9). In silver nanoparticle containing articles, the antimicrobial activity is also sustained for 4 days as evident from the results in Table 10. In

the case of some substrates such as fiber, catheter and lens, the antimicrobial activity was tested by the bacterial challenge test. In such a test, the substrates are challenged with known bacterial count while immersed in medium for 24h. The medium was then appropriately diluted and plated on MHA plates to estimate the surviving bacterial count. The challenges were continued until the substrates are exhausted of an effective amount of silver. The bacterial challenge test results (Table 11) show that silver ions release from nanoparticles embedded in substrate surface occurring over 11 challenges i.e. 11 days. In contrast, similar commercial products (Bardex & Lubrisil I.C. catheters) lasted only 3 days.

Table 10: Examples of Serial Transfer Results Against *Staphylococcus Aureus*

Example	Substrate	Day 1	Day 2	Day 3	Day 4	Day 5
A6	Contact lens	13.5/6.5	9.0/6.5	7.0/6.5	6.5/6.5	-
B1	Hyd.polymer	13.5/5.5	8.5/6.0	6.0/5.5	-	-
B2	Hyd.polymer w/ copper	12.0/5.0	10.0/5.0	8.0/5.0	7.0/5.5	5.5/5.5

Biocompatibility of medical devices with tissues is important. The agarose overlay assay is used to quantify the inherent level of cytotoxicity present in device. The results from agarose overlay tests verified that silver nanoparticle containing substrates are non-cytotoxic as well as non-irritating. The sonication of silver treated nylon fiber had no effect on antimicrobial activity and repeatedly washing of the gauze did not result in loss of activity. The results summarized here clearly demonstrate that liquid compositions containing silver nanoparticles are stable, can be made very easily and cheaply and can be used to make a host of devices' surfaces antimicrobial.

In general, the present invention comprises compositions comprising nanoparticles. Nanoparticle compositions comprise a solvent, a silver nanoparticle, and a stabilizing agent. After formation of the nanoparticles, there may be residual or unreacted reducing agent remaining in the composition. It is understood that a large number of nanoparticles form in the composition. The solution may aqueous or non-aqueous. Aqueous solvents include water, and non-aqueous solvents include methylene chloride, chloroform other aliphatic and aromatic chlorinated solvents, cyclohexane, diethyl ether, ethyl acetate and mixtures thereof, stabilizing agents, stabilizers, or other similar terms, which are used interchangeably include a polymer, a surfactant or both. Polymers include a homopolymer copolymer, synthetic or naturally derived, polymers of acrylamide and its derivatives, methacrylamide and its derivatives, polyamides, polyurethanes, polymers having no particular backbone but with urethane segments or tertiary amine groups in the side chains, other polymers predominantly polar in nature or co-polymers

having a portion that is derived from polar co-monomers, methaacrylamide, substituted acrylamides, substituted methaacrylamides, acrylic acid, methacrylic acid, hydroxyethyl methacrylate, acrylonitrile, 2-acrylamido-2-methylpropane sulfonic acid and its salts (sodium, potassium, ammonium), 2-vinyl pyrrolidone, 2-vinyl oxazoline, vinyl acetate, maleic anhydride. Surfactants may be anionic, nonionic, or amphoteric surfactants.

Methods of making silver nanoparticles comprise a) adding in no particular order, an aqueous solution of a stabilizing agent solution, an anionic donating solution and a soluble silver salt solution, and b) adding a tertiary diamine solution, and further c) heating the final solution to increase the reaction. The method further comprises forming the nanoparticles in situ on the surface of an article. The articles may be a woven or nonwoven fiber article article. The article may be a medical device, polymer, a fiber, a metal, glass, ceramic, fabric or combination thereof.

The nanoparticles may be extracted into a non-aqueous solution. The invention also comprises methods of treating a surface with silver nanoparticles, comprising, a)contacting a surface with a solution comprising silver nanoparticles for a time sufficient for an effective amount of nanoparticles to bind to the surface, and b)rinsing the solution from the surface. The steps of contacting and rinsing may be repeated multiple times to increase the number of nanoparticles adhering to the surface. The surface contacted may be a medical device or any of the other articles or surfaces taught herein. The method further comprises, contacting the surface with nanoparticles adhered thereto with an aqueous solution of hydrogen peroxide for a sufficient period of time, and, rinsing the hydrogen peroxide solution from the surface, wherein the surface contacted may be a medical device, polymer, a fiber, a metal, glass, ceramic, fabric or combination thereof.

The present invention comprises methods of rendering an elastomeric surface electrically conductive, comprising, a) contacting an elastomeric surface with a solution comprising metal nanoparticles for a time sufficient for an effective amount of the nanoparticles to adhere to the surface, and b)rinsing the surface. Such elastomeric surfaces may optionally be reflective. Such elastomeric surfaces may be reflective and not electrically conductive. Metal nanoparticles used in such methods may be made by methods comprising, a) adding in no particular order, an aqueous solution of a stabilizing agent solution, an anionic donating solution and a soluble metal salt solution, and b) adding a reducing solution. The metal nanoparticle may comprise silver, gold, platinum, iridium, rhodium, palladium, copper or zinc. The method for making the metal nanoparticle may further comprise heating the final solution. The contacting and rinsing steps

may be repeated multiple times to increase the number of nanoparticles adhering to the surface. The surface contacted may be silicone, polyurethane, synthetic or natural rubber, a synthetic or natural polymer, flexible polymers of polyimides, polyamides, polyacetals, polysulfones, PBTs, PBO's, ethylene and propylene based polymers, acetate polymers, polyacrylates, polycarbonate, PET's, PEN's or blends thereof or co-polymeric derivatives. The elastomeric or flexible surfaces may be further treated by c) contacting the elastomeric surface with nanoparticles adhered thereto with an aqueous solution of hydrogen peroxide for a sufficient period of time, and, d)rinsing the hydrogen peroxide solution from the surface.

The present invention comprises elastomeric surfaces and articles made by such methods, wherein an article produced by a method of rendering an elastomeric surface electrically conductive or optionally, wherein the method comprises a) contacting an elastomeric surface with a solution comprising metal nanoparticles for a time sufficient for an effective amount of the nanoparticles to adhere to the surface, and b) rinsing the surface. Such articles or surfaces may comprise flexible mirrors, stretchable elastic conductive polymers, articles used to reduce electromagnetic interference, to shield devices and circuits against electrostatic discharging, and to impart radar invisibility to aircraft or other vehicles.

The present invention comprises methods of rendering an article or surface contacting a fluid resistant to biofilm formation, comprising, a) contacting the article or surface with a solution comprising metal nanoparticles for a time sufficient for an effective amount of the nanoparticles to adhere to the surface, and b) rinsing the surface. Metal nanoparticles used in such methods may be made by methods comprising, a) adding in no particular order, an aqueous solution of a stabilizing agent solution, an anionic donating solution and a soluble metal salt solution, and b) adding a reducing solution. Metal nanoparticles used in such methods may be made by methods comprising, a) adding in no particular order, an aqueous solution of a stabilizing agent solution, an anionic donating solution and a soluble metal salt solution, and b) adding a reducing solution. The metal nanoparticle may comprise silver, gold, platinum, iridium, rhodium, palladium, copper or zinc. The method for making the metal nanoparticle may further comprise heating the final solution. The contacting and rinsing steps may be repeated multiple times to increase the number of nanoparticles adhering to the surface. The article or surface contacting a fluid that is contacted by the nanoparticles may be made of steel, stainless steel, glass, titanium, copper, gold, synthetic and natural polymers, polypropylene, polycarbonate, polyurethane, polyvinyl chloride, polystyrene, polysulfone, silicones, HTV, RTV, blends or co-polymeric derivatives. The article or surface contacting a fluid to be made resistant to biofilm

formation may be further treated by c) contacting the article or surface contacting a fluid with nanoparticles adhered thereto with an aqueous solution of hydrogen peroxide for a sufficient period of time, and, d)rinsing the hydrogen peroxide solution from the surface. The present invention also comprises articles produced by rendering an article or surface contacting a fluid resistant to biofilm formation, wherein the method comprises a) contacting an article or surface contacting a fluid with a solution comprising metal nanoparticles for a time sufficient for an effective amount of the nanoparticles to adhere to the article or surface, and b) rinsing the article or surface. Such articles include, but are not limited to, food storage and preparation devices, laboratory equipment, marine or water vehicles, hulls, propellers, anchors, ballast tanks, motors, pilings, liquid filtering equipment, tubing, ropes, chains, fish tanks, liquid containers, water bowls, cooling towers, water tanks, canteens, fuel tanks, or storage bins.

The present invention comprises methods of making metal nanoparticles comprising, a) adding in no particular order, an aqueous solution of a stabilizing agent solution, an anionic donating solution and a soluble metal salt solution, and, b) adding a reducing solution. The stabilizing agent solution comprises a surfactant, a polymer or both. The polymer is a homopolymer copolymer, synthetic or naturally derived, polymers of acrylamide and its derivatives, methacrylamide and its derivatives, polyamides, polyurethanes, polymers having no particular backbone but with urethane segments or tertiary amine groups in the side chains, other polymers predominantly polar in nature or co-polymers having a portion that is derived from polar co-monomers, methacrylamide, substituted acrylamides, substituted methaacrylamides, acrylic acid, methacrylic acid, hydroxyethyl methacrylate, acrylonitrile, 2-acrylamido-2-methylpropane sulfonic acid and its salts (sodium, potassium, ammonium), 2-vinyl pyrrolidone, 2-vinyl oxazoline, vinyl acetate, maleic anhydride. The metal nanoparticles may be formed in situ on a surface or the surface of an article. The nanoparticles may be extracted into a non-aqueous solution. The present invention also comprises metal nanoparticles made by such methods.

It must be noted that, as used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise.

All patents, patent applications and references included herein are specifically incorporated by reference in their entireties.

It should be understood, of course, that the foregoing relates only to exemplary embodiments of the present invention and that numerous modifications or alterations may be

made therein without departing from the spirit and the scope of the invention as set forth in this disclosure.

Although the exemplary embodiments of the present invention are provided herein, the present invention is not limited to these embodiments. There are numerous modifications or alterations that may suggest themselves to those skilled in the art.

The present invention is further illustrated by way of the examples contained herein, which are provided for clarity of understanding. The exemplary embodiments should not to be construed in any way as imposing limitations upon the scope thereof. On the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention and/or the scope of the appended claims.

Examples

Antimicrobial Device Examples 1-37

Example 1 Cotton gauze

Dimethyl formamide (5 ml) was heated in beaker to ~ 60C under stirring. After the stir bar was removed a 2"x2" cotton gauze (Curity brand, The Kendall Company, Mansfield, MA) was placed in DMF to soak up all solvent. Silver nitrate solution (0.3 ml, 0.1M) was pipetted over the gauze. Within a minute the gauze turned yellow. After 5 minutes, the beaker was removed from the hot plate and cooled to room temperature. The pale yellow colored gauze was thoroughly rinsed with de-ionized water, blotted and dried in oven at 40C.

Table 9: Examples of Sustained Release of Silver from Bacterial Challenge Test Against *Pseudomonas Aeruginosa* ATCC 9027(Each challenge is 24h)

% Kill Rate of *Pseudomonas Aeruginosa*

Challenge No.	Inoculation size (cfu/ml)	Example 15	Example 16	Example 14	Example 13
1	6300	100	100	100	100
2	4600	100	100	100	100
3	8700	100	100	100	100
4	3000	66.67	100	100	100
5	7000	100	0	100	97.14
6	8000	100	0	100	100
7	4000	100	Stopped	100	100
8	7000	100		94.14	57.14
9	5000	100		100	100
10	9000	100		100	100
11	4000	100		100	100
12	8000	54.88		0	0
13	6000	0		0	0

Bio-film Inhibition Test

For in-dwelling medical devices such as urinary or venous catheters, having antimicrobial surface characteristics is very helpful for minimizing infections. But, even more important is the ability of such devices to prevent bio-film formation. Once bacteria have formed bio-films, they use it as shield making it difficult to get rid of them. Antibiotics or other drugs are not effective. One important distinguishing feature of the antimicrobial devices of the present invention is their ability to inhibit bio-film formation. To examine the bio-film inhibition characteristics of the antimicrobial nylon tubing, a method based on following principle was employed.

Bio-film formation can be evaluated by immersing the test article in test medium that has been inoculated with the challenge organism. After appropriate incubation, bio-film formation

is assessed by determining the amount of carbohydrate specific dye that is bound on the surface of the device. There is a quantitative relationship between the extent of bio-film formation and residual carbohydrate on the surface. This can be quantified by first extracting the dye in a suitable solvent and then measuring the OD on a spectrophotometer.

Figure 17 summarizes the results of bio-film testing on nylon tubing samples with silver loading (in the form of nanoparticles) of ~ 600 ppm (based on the tubing weight). The silver treated samples strongly inhibit bio-film formation against, *E. Coli*, methicillin resistant *staphylococcus aureus*, *pseudomonas aeruginosa* and *candida albicans*. In comparison, untreated device samples show no inhibition (high OD values). The results unequivocally show the resistance of the device of the present invention to bio-film formation.

Example 2 Cotton gauze

Gauze was treated exactly as in example 1 except the silver nitrate solution concentration was 1.0M.

Example 3 Contact lens

Contact lens (SEE3, CibaVision Corporation, Duluth, GA) was rinsed clean off the preservative solution and immersed in hot DMF solution as in example 1. Under gentle stirring, silver nitrate (0.3 ml, 1.0M) was added drop-wise to the hot DMF. After 5-7 minutes, the beaker contents were cooled, lens removed and rinsed thoroughly with de-ionized water, blotted over tissue paper and dried in oven at 40° C. The lens imparted pale yellow tint.

Example 4 Catheter segment

DMF solvent (10 ml) was heated to ~ 100° C in a beaker under stirring. Silver nitrate solution (0.25ml, 0.02M) was added to the hot solvent to yield silver nanoparticles as indicated by yellow color (due to plasmon resonance band). A pre-cleaned silicone catheter (14 Fr, Degania Silicone Ltd, Israel) segment ~ 1" long was immersed in the yellow solution for 15 minutes. The catheter segment was removed, rinsed with de-ionized water and dried. A small level of discoloration of the catheter segment was seen.

Example 5 Hydrogel sheet – Method 1

To de-ionized water (13.3ml) in a cup, acrylamide (1.482 g), bisacrylamide (0.018 g) and glycerol (1.5 g) were added under stirring. Separately, in hot (~ 60° C) de-ionized water (10 ml), isopropanol and guar gum (0.165 g) were dissolved and the solution was allowed to cool to room temperature. The guar gum and acrylamide monomer solutions were mixed. To the mixture, silver nitrate (1 ml, 0.1M) and sodium saccharinate (1 ml, 0.125M) were added. With

the help of a spatula, the viscous mass was mixed. Upon precipitation of silver saccharinate, the viscous mass turned whitish opaque.

To the silver salt containing mass, ammonium persulfate (0.05 g dissolved in 1 ml of water) was added followed by TEMED (0.063 ml in 1 ml of water). After TEMED addition, the mass began to slowly turn brown colored with no immediate polymerization. After 8 days, the viscous mass had converted into a brown colored hydrogel sheet.

Example 6 Contact lens

Contact lens (SEE3 brand, CibaVision Corporation, Duluth, GA) was rinsed with de-ionized water to rinse off the preservative solution and then it was soaked with the silver nitrate solution (0.15 ml, 0.1M) for 10 minutes. Excess solution was drained off and sodium saccharinate (0.15 ml, 0.125M) was added to re-immerse the lens. Lens turned opaque due to the in-situ formation of silver saccharinate. Excess liquid and any loose solids were pipetted off and the lens rinsed once again with de-ionized water. TEMED (0.1 ml) mixed with water (0.2 ml) were added to soak the lens and initiate reduction. After 5 minutes, the liquid turned pale yellow. At that point, all liquid was discarded and the lens rinsed several times with water and dried overnight under ambient conditions.

Example 7 Nylon fiber

Several strands of fibers (~ 1 mm dia) made of nylon (polyamide) were immersed in silver nanoparticles composition made in example B6 (total liquid volume 10 ml) for 72 hours at room temperature. The immersed fibers were rinsed thoroughly with 70% aqueous IPA and water. The fibers were also gently wiped with tissue soaked in IPA and dried for 15 minutes at 45° C. The soaked portion of the fibers was colored light yellow to brown.

Example 8 Silicone catheter segment

4" long 14 Fr silicone catheter segment (Degania Ltd, Israel) was cleaned with IPA and dried. The segment was dipped in 5 ml THF solution of saccharin (0.5gm) for 1h. The shaft was removed and rinsed quickly with acetone once and immersed in silver nitrate solution (0.5 g silver nitrate, 5 ml 90% acetone/water) for 0.5h. The shaft segment was removed and thoroughly rinsed with water and finally dipped in 30% TEMED solution in IPA. The solution was warmed to induce reduction and set aside overnight. The segment had turned yellow indicating reduction reaction had progressed. The shaft was rinsed with water and dried in oven at 125° C to remove all traces of TEMED.

Example 9 Catheter with hydrophilic polymer coating

A small catheter segment ~ 3" long with hydrophilic polymer coating (2.7% GRAFT-COAT, STS Biopolymers, Henrietta, NY) was immersed in silver nanoparticles solution prepared in a manner of example B4 for 2h. The segment was removed and washed with water and dried at 45° C. Barely any color was seen initially but after several days a uniform brown color developed in the coating.

Example 10 Contact lens

Single lens (SEE3, CibaVision Corporation) was soaked in 7 ml of the stock solution prepared in example B7 at room temperature for 12-16h. The lens was rinsed with water and dried at room temperature. The lens was covered with a uniform shiny transparent silver coating.

Example 11 Cotton gauze

Cotton gauze (Curity brand, The Kendall Company, Mansfield, MA) about 3"x3" in size was successively soaked in silver nitrate (0.1M) and sodium saccharinate (0.125M) with blotting after each soak and dried at 110° C for 10 minutes. The dried gauze with silver salt was re-soaked in 30% TEMED solution in IPA for 72h, rinsed thoroughly with water, left to soak for 24h in water to remove solvent traces and dried. The gauze turned yellow after about 3h soak in TEMED. The color did not leach during the rinsing and water soak steps.

Example 12 Cotton gauze

Cotton gauze identical to the one in example 15 was soaked in PAA-silver nanoparticles solution (5 ml) prepared in a manner of example 40 for 72h. The gauze was rinsed with water and left to soak in water for 24h and dried. The gauze imparted orange yellow shade and did not leach any color during rinsing and water soak steps.

Example 13 Contact lens

Clear contact lens with embedded silver nanoparticles was prepared as follows. Silver nanoparticles containing composition was prepared by dissolving Tween 20 in water (1 ml), followed by the addition of sodium saccharinate (1 ml, 0.125 M), silver nitrate (1 ml, 0.1M) and TEMED (0.1 ml). The solution (0.5 ml) after aging for a week was diluted to 2 ml with water and a pre washed contact lens was immersed in it overnight. The lens was washed with water, gently blotted and dried in oven at 75° C for 0.5h.

Example 14 Silicone catheter

16 Fr Silicone catheter segment (~ 6" long) was washed with isopropyl alcohol (IPA) and dried. It was soaked in THF for 1h to cause swelling of its walls and then dipped overnight

in 1 week old silver nanoparticles solution prepared as follows. Tween 20 (0.025 g) was dissolved in sodium saccharinate solution (5 ml, 0.125M) and silver nitrate (5 ml, 0.1M) and 0.5 ml TEMED added to it. The resulting liquid was briefly heated (10s) in microwave oven causing the solution to become yellow brown. After overnight soak, the catheter was rinsed with water, IPA and water again and dried in oven.

Example 15 Nylon catheter – Method 1

Nylon catheter piece ~ 1mm dia, 15" long (IFLOW Corporation, Lake Forest, CA) was cleaned with IPA and wiped dry. Catheter was soaked overnight in silver nanoparticles stock solution (90 ml) prepared according to the procedure of example 44, washed with water, IPA and wiped dry and further dried in oven at 45° C. After treatment, the catheter imparted a shade of yellow.

Example 16 Nylon Catheter – Method 2

Nylon catheter segment ~ 4" long but otherwise similar to example 15 was briefly (1 minute) dipped in THF solution of γ-aminopropyl triethoxy silane (0.1 ml silane/5 ml THF), removed and dried in air for few minutes. The silane coated sample was soaked in freshly prepared silver nanoparticles stock solution (example 44) overnight. The catheter segment was washed with water, IPA and wiped dry. The sample imparted more uniform and intense yellow color than sample of example 15.

Example 17 Silicone catheter – Bard

Catheter segment ~ 3" long (Lubrisil brand, BARD Inc. Covington, GA) was wiped with IPA and soaked overnight in silver nanoparticles stock solution prepared by method of example 14. The segment was rinsed with water, IPA and dried in oven at 45° C. It imparted pale yellow brown color.

Example 18 Silicone breast implant membrane

3 pieces (~ 1"x1") of breast implant membrane (~ 0.5 to 1 mm thick) made of silicone were impregnated with silver nanoparticles by first swelling it according to the step in example 14 and soaking it overnight in silver nanoparticles solution made by the method of example 44. The pieces were washed washed with water, IPA and dried in oven at 75° C for few hours. Each piece after treatment imparted pale yellow shade.

Example 19 Cyotoxicity of nylon fiber strands

A silver nanoparticles solution was first prepared by mixing 0.2gm Tween 20 in 4 ml water, adding 4 ml sodium saccharinate (0.125M), then 4 ml silver nitrate (0.1M) followed by 0.4 ml TEMED and heating in microwave oven (1500W power) for 10 seconds and then cooling

to room temperature. Four nylon fiber strands (~ 1mm dia & 9" long) were immersed in the solution overnight. The strands were rinsed with water several times and dried in air. After silver nanoparticles impregnation, the fiber surface impart yellow brown color.

Using agarose overlay no cytoxicity to L929 fibroblast cells was observed. The silver content of the fiber was ~ 800 ppm.

Example 20 Cyotoxicity of silicone catheter of Example 14

Using agarose overlay no cytoxicity to L929 fibroblast cells due to the silver treated catheter was observed. The silver content of the catheter was estimated to be greater than 800 ppm.

Example 21: Effect of Sterilization Methods on Substrates with Silver Nanoparticles

Silicone catheters of Example 14 and nylon fiber strands of Example 19 were subjected to ethylene oxide (ETO) sterilization. The samples saw ETO dose typical of high volume products such as medical tubings and kits. After sterilization there was a small visually detectable change after sterilization. Both samples turned slightly darker than the original shade.

Examples 22 Attempt to "bleach" yellow color of silver gauze comprising silver nanoparticles

Several pieces (3"x3") of Curity (Kendall) cotton gauze were dripped with 2 ml each of a solution comprising silver nanoparticles prepared according to the following manner: 10ml each of stock solutions of Tween 20 (concn: 50 gm/L), sodium saccharinate (0.125M) and silver nitrate (0.1M) were mixed on vortex mixer and TEMED (1 mL) was added. The resulting solution was heated in a microwave oven for 30 seconds to yield a yellow brown solution that was cooled to room temperature.

The gauze pieces were blotted and dried in oven at 45C overnight. Upon drying some gauze color changed to light brown. The gauzes were soaked in 10% hydrogen peroxide solution (25 mL). Not color change was observed in first few minutes though after more than an hour the brown color much lighter. After 24h soak, the gauze pieces had turned white. They were blotted and dried in oven at 45° C for 1 hour and left under lab light for continuous exposure for 36h. Except slight discoloration in few spots, the gauzes looked unchanged giving us another method of preparing silver antimicrobial gauze material.

Examples 23 Impregnation of silicone catheter by treatment with non-aqueous silver nanoparticles composition

An aqueous composition similar to the one in example 50 was made and left undisturbed for over a week in a capped vial. The composition was diluted with 25 mL deionized water and extracted with ~ 15 mL chloroform. A portion of silver nanoparticles were

extracted into the chloroform layer. A clean catheter stem made of silicone (14Fr, Degania Ltd, Israel) was dipped into chloroform layer for 0.5h. Immersed portion of catheter swelled due to solvent absorption. The catheter was then removed and without rinsing dried in oven at 45C for 15-20 minutes. Following treatment, it imparted faint yellow color that after 24h turned to orange red. The color change indicated the presence of silver nanoparticles in the catheter walls. It was found to antimicrobial in 24h bacterial challenge test.

Example 24 Silver treated PTFE

10 ml each of stock solutions of Tween 20 (concn: 16.7 gm/L), sodium saccharinate (0.125M) and silver nitrate (0.1M) were mixed on vortex mixer and TEMED (1 mL) was added. The resulting solution was heated in a microwave oven for 60 seconds to yield a yellow brown solution. PTFE thread seal tape 4" long was wrapped around a test tube and then this tube and placed inside a large test tube and the silver nanoparticle solution was poured in both tubes to submerge the tape for 24h and maintained at 55° C. The tape was rinsed thoroughly with water several times and dried for 0.5h at 55° C. After silver nanoparticles impregnation the tape imparted pale yellow color. It was found to be antimicrobial in a 24h bacterial challenge test.

Example 25 Silver treated PP

10 ml each of stock solutions of Tween 20 (concn: 16.7 gm/L), sodium saccharinate (0.125M) and silver nitrate (0.1M) were mixed on vortex mixer and TEMED (1 mL) was added. The resulting solution was heated in a microwave oven for 60 seconds to yield a yellow brown solution.

PP strip were surface treated to improve aqueous wettability as follows: 4 polypropylene strips (3"x ¼") were soaked in a 80 mL 9M sulfuric acid under stirring for 40h. Thereafter, the strips were rinsed with water several times and patted dry on paper and then air dried. Next, the strip were placed in a THF solution of g-aminopropyl triethoxysilane made by adding the silane (0.2 mL), 0.1 mL water and 0.1 mL boron trifluoride etherate to 10 mL THF. After soaking for 5 minutes, the strips were removed and air dried briefly and then at 55° C for 0.5h.

The silane treated strips were then immersed in silver nanoparticles solution made above for 4h, rinsed, blotted on paper and air dried. Each strip imparted pale yellow color indicating impregnation of silver nanoparticles.

Example 26 Effect of <1 ratio of Sac/Ag on deposition of Ag on nylon fibers

Tween 20 solution (3 mL, 16.7 g/L), sodium saccharinate (3 mL, 0.025M) and silver nitrate (3 mL, 0.1M) were vortexed together. TEMED (0.1 mL) was added and vortexed again. TEMED addition turned the mixture pale yellow. The solution was briefly heated in microwave

to ~ 55C and 4 clean nylon fiber strands were immersed in the hot solution for 4h. The immersed portion of the fibers had turned blue black. The fibers were cleaned thoroughly and dried. The fibers were found to be antimicrobial in ZOI assay.

Example 27 Silver treated polysulfone

Tween 20 solution (2 mL, 16.7 g/L), sodium saccharinate (2 mL, 0.125M) and silver nitrate (2 mL, 0.1M) were vortexed together. TEMED (0.2 mL) was added and vortexed again. The solution was briefly heated in microwave to ~ 70-75C cooled to 55° C and then seven 6" pieces of hollow polysulfone tubes (< 0.5 mm dia) were immersed in the hot solution for 4h. The tubes were rinsed with water and centrifuged with the tubes immersed in water to clean them from the inside. The white polysulfone tubes had become yellow colored and in ZOI assay were found to be antimicrobial.

Example 28 Method of depositing silver on fabrics by treatment with fumarate based composition of example B33 and acetic acid

Several cotton gauze pieces (2"x2" from Bulkee II gauze roll) are treated with the silver nanoparticles composition made in example 70 by soaking in the composition for few minutes, followed by blotting and then re-soaking them in dilute acetic acid (5 ml glacial acetic acid in 100 mL water) for few minutes to precipitate out the silver nanoparticles stabilized with fumarate. After blotting on paper and drying in oven at 55° C for 0.5h, gauzes with silver are obtained as light yellow colored material. The gauzes are expected to be antimicrobial.

Example 29 Effect of ammonia on catheters made from PEBEX® nylon tubing stock

Silver nanoparticles impregnated catheters tubing pieces (2 pieces 2" long, 1mm outer diameter and 0.6 mm inside diameter, made from tubing stock of PEBEX® grade polyamide polymer) were soaked in dilute ammonia solution (2 mL 28% ammonia in 8 mL water) in a test tube to examine if the silver nanoparticles can be dissolved away. No change was observed in color after 16h suggesting no effect of ~ 7 % ammonia on silver nanoparticles impregnated on a surface.

Example 30 Silver treated PVC drain

Polyvinyl chloride (PVC) tubing several feet long having ¼" OD was soaked overnight in silver nanoparticles solution prepared from Tween 20 solution (160 mL, 16.7 g/L), sodium saccharinate (160 mL, 0.125M) and silver nitrate (160 mL, 0.1M) after mixing in succession and stirring together for 15 minutes. TEMED (16 mL) was added and stirred. The solution was heated in microwave to ~ 70-75C cooled to 55° C. The tubing was removed and quenched in

de-ionized water, rinsed in running water and air dried. The tubing colorless before treatment yellow and was uniform in color. It was found to be antimicrobial in bacterial challenge test.

Example 31 Silver treated PEBEX® grade nylon tubing catheters –conditions versus ppm

This example examines the effects of time, starting concentration of silver nitrate and temperature on the amount of silver deposited on small dia nylon tubing material made of PEBEX® type of nylon grade. The tubing simulates a type of material used in catheters. The tubing was comprised of ~ 1 mm OD and 0.6 mm ID and came 27" in length.

Tween 20 solution (160 mL, 16.7 g/L), sodium saccharinate (160 mL, 0.125M) and silver nitrate (160 mL, 0.1M) were mixed in succession and stirred together for 15 minutes. TEMED (16 mL) was added and stirred. The solution was heated in microwave to ~ 70-75° C cooled to 40-45° C. A dozen or so catheter pieces were placed in a pyrex dish and weighed down (to prevent them from floating). The cooled silver nanoparticles solution was poured over the catheters in the dish and one catheter was removed at a given time point, thoroughly cleaned and air dried. The nylon tubing imparted yellow color of increasing intensity with time. The tubing samples were analyzed for silver content by AAS.

The test was repeated at 55-60° C by cooling the solution to that temperature before pouring it on the cathetersThe silver content (as average of 3 portions- top, middle and bottom) of the catheter) as function of the time of treatment at two temperatures are tabulated in Table12.

Table 10: Silver Content of Nylon Tubing in ppm

Treatment time(h)	T ~ 40-45° C	T~ 55-60° C
0.25	51	110
1	122	230
2	130	440
4	179	1017
8	290	1897

Example 32: Effect of silver concentration on loading on the nylon tubing material

To study the effect of concentration, the starting concentration of silver nitrate in preparing the treating solution was varied. For this experiment radioactive silver was used and counts determined the silver content instead of AAS assay technique.

Briefly, Tween 20 solution (13.3 mL, 16.7 g/L), sodium saccharinate (1.3 mL, 0.125M) and 1.3 mL ^{110m}Ag silver nitrate (in different concentrations), water (24 mL) were mixed in succession and stirred together for 15 minutes. TEMED (0.13 mL) was added and stirred. The solution was heated in microwave to ~ 70-75° C cooled to 52° C. To the solution were added 33

pieces of tubing material 2 cm in length and centrifuged briefly to remove air bubbles and incubated at 52° C for 16 hours. The catheters were thoroughly rinsed clean and air dried.

From the counts measured and specific activity, the amount of silver deposited on the tubing was determined. The results are presented below in Table13.

Table 13: ^{110m}Ag loading in nylon tubing samples

Sample No.	AgNO3 in treatment solution (g/L)	Ag content in tubing (ppm) (n=5)
1	0.755	1422
2	0.670	1330
3	0.548	1235
4	0.426	1019
5	0.296	876

Example 33 Silver treated nylon tubing – effect of nitric acid

A catheter nylon tubing (1 mm OD) made of PEBEX having silver loading of ~ 920 ppm was prepared by following procedure of Example 31. The amber colored catheter piece 1" long was immersed in 5 ml dilute nitric acid (prepared from 0.5 mL tech grade nitric acid and 4.5 mL water) overnight. The piece was washed with de-ionized water twice, then with isopropanol and dried by blowing nitrogen gas. After acid treatment, the piece was bleached to faint yellow. Silver analysis by AAS showed a loading of 350 ppm indicating a reduction of ~ 62% from the original loading.

This example affords a method of altering the silver loading of silver nanoparticles impregnated articles by treatment with nitric acid if the actual loading exceeds a proposed target.During the test, discoloration (indicating loss of silver) of the substrate due to exposure to nitric acid vapors was observed. This result affords a method to pattern a silver nanoparticles bearing surface by exposing them to nitric acid vapors or of other acids possessing similar characteristics.

Example 34 Silver treated nylon tubing – effect of H_2O_2

The nylon tubing samples deposited with ^{110m}Ag after the egress experiment of example 32 were in this example for studying the effect of H_2O_2 to eliminate the amber color from the tubing surface. Just before soaking the sample tubings in H_2O_2, the silver loading in ppm was determined by measuring the radioactivity. The samples in separate tubes were then soaked in 2 mL 30% H_2O_2 solution for 24 hr at ambient temperature. Bubble formation due to oxygen was observed at the tubing surfaces often floating the tubing pieces. The next day, all samples had changed in color from amber to colorless. The radioactivity of the samples was again measured and from the specific activity, the silver loading was calculated. The results given below (Table

14) indicate the silver loss due to peroxide treatment is equivalent to the loss during 24h saline soak. The amber color silver nanoparticle comprising surfaces become colorless without loss of silver (or antimicrobial activity).

Table 14: ^{110m}Ag content of nylon tubing samples before and after H_2O_2 treatment

Sample No.	$AgNO_3$ in original treatment solution (g/L)	Ag content in tubing (ppm) (n=5) beforeH_2O_2	Ag content in tubing (ppm) (n=5) afterH_2O_2
1	0.755	1181± 9	1173 ± 10
2	0.670	1095 ± 3	1088 ± 4
3	0.548	1015 ± 3	1009 ± 4
4	0.426	800 ± 6	795 ± 7
5	0.296	700 ± 5	696 ± 5

Example 35: Antimicrobial metal implants

10 mL each of Tween 20 surfactant solution (16.7 g/L), sodium saccharinate (0.125M), silver nitrate and 20 mL de-ionized water were mixed under stirring in a beaker to yield a suspension with white particles. To the suspension, TEMED (1.5 mL) was added and briefly mixed. The content was heated for a minute in a microwave oven and the hot solution was poured on three metal implant parts placed in a glass petri-dish. The dish was covered and heated to 70° C for 4 hours. Metal parts were removed from the solution, rinsed with de-ionized water several times, placed in a beaker with water and ultrasonicated for 15 minutes to remove loose particles. The metal parts were then dried in air. The implant with silver nanoparticle impregnated surface showed antimicrobial activity against pseudomonas that sustained for 3 days. In contrast, untreated control metal part showed uncontrolled bacterial growth.

Example 36: Antimicrobial polyurethane foams

Antimicrobial silver nanoparticle composition was prepared by mixing 25.5 mL each of Tween 20 solution (5.2 g/L), sodium saccahrinate (0.0125M) and silver nitrate (0.01M) followed by TEMED (0.255 mL) addition and heating the mixture at 48° C for 16h. The cooled solution was used in the preparation of foams. 1" squares of Supersoft S00-T foam from Lindell Manufacturing from Michigan and Medical grade (Type 562-6) from Rynel Corporation of Maine were soaked in the silver nanoparticle compositions and blotted lightly and dried in oven at 45° C for 0.5h. The foams were found to be antimicrobial in a ZOI assay against *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Example 37 Antimicrobial silicone catheter stems – effect of different sterilization processes

Several stems of isopropyl alcohol cleaned silicone catheter (14 Fr, Degania Silicone Ltd., Israel) were soaked in THF for a period of 15-30 minutes. Separately an antimicrobial silver nanoparticle composition was prepared by mixing equal volumes of Tween 20 (50 g/L),

sodium saccharinate (0.125M) and silver nitrate (0.1M) and then adding TEMED ($1/10^{th}$ the individual stock solution volume). The resulting mixture was briefly heated in microwave oven for 30 to 45 seconds until the solution turned yellow. The solution was cooled to room temperature and then catheter stems swollen in THF were placed in the silver nanoparticle solution overnight to deposit the particles on the silicone catheter surface. The stems were thoroughly rinsed with water and dried in air. After silver impregnation the color changed to yellow brown to grey brown. A few stems with silver nanoparticles each were sterilized by steam sterilization at 122° C for 15 minutes, e-beam process (approx 30 kGy) and commercial standard ETO process. Sterilized catheter stems with silver were found to be equally antimicrobial over 7 bacterial challenges (24h) of *Pseudomonas aeruginosa* strains with inoculation dose ~ 5e3 cfu/mL with 100% kill rate. None of the sterilization processes studied had adverse effect on the antimicrobial property of the catheters.

Example 38 Hydrophilic cross-linked polymer

To de-ionized water (13.3ml) in a cup, acrylamide (1.482 g), bisacrylamide (0.018 g) and glycerol (1.5 g) were added under stirring. To the mixture, silver nitrate (1 ml, 0.1M) and sodium saccharinate (1 ml, 0.125M) were added. Upon precipitation of silver saccharinate, the resulting liquid turned whitish opaque.

To the silver salt containing mass, ammonium persulfate (0.05 g dissolved in 1 ml of water) was added followed by TEMED (0.113 ml in 1 ml of water). After TEMED addition, the mass began to slowly turn brown and was set aside overnight to polymerize to yield red brown colored brittle solid polymer.

Example 39 Copper modified hydrophilic cross-linked polymer

A portion of solid polymer (~ 0.1 g) from Example 38 and cupric chloride solution (1 ml, 0.1M) were placed in a capped vial and set aside several days. The brown color of the polymer had changed to blue due to hydration by cupric chloride solution and the conversion of the nanoparticles to silver chloride.

Example 40 Hydrogel sheet – Method 2

A silver nanoparticles containing polymer solution was prepared as follows. Acrylamide (0.5 gm) was dissolved in de-ionized water (5 ml). To the solution under mixing, ammonium persulfate (16 mg) and TEMED (0.02 ml) were added to form polyacrylamide (PAA) polymer solution. In the PAA solution diluted first with 5 ml water, silver saccharinate was precipitated by successively adding sodium saccharinate (1 ml, 0.125M) and silver nitrate (1 ml, 0.1M). Silver nanoparticle formation by reduction was initiated by adding TEMED (0.05 ml) to the

PAA solution (indicated by the solution turning red brown). If needed, the solution was warmed to initiate reduction reaction. The solution was set aside for at least 1 day to complete the reduction.

To the PAA – silver nanoparticles solution prepared above, acrylamide (1.482 g), bisacrylamide (0.018 g) and glycerol (1.5 g) were added under stirring. Separately, to hot (~ 60° C) de-ionized water (10 ml), isopropanol and guar gum (0.165 g) were added to form solution that was cooled to room temperature. The guar gum and the PAA-silver nanoparticles monomer solution were mixed. To the mixture, hydrogen peroxide solution (2 ml, 10%) was added causing the solution to pale from its original red brown color. Soon after adding the initiator, ammonium persulfate (0.05 g), the monomer solution with silver nanoparticles formed a red brown gel. The gel was transferred to a petri-dish and left to dry overnight.

Example 41 Talc powder

A silver nanoparticles containing composition was prepared as follows. Surfactant Tween 20 (0.05 g) was dissolved in water (2.5 ml). To the surfactant solution, sodium saccharinate (0.25 ml, 0.125M), silver nitrate (0.25 ml, 0.1M) and TEMED (0.1 ml) were added one after another. The mixture was heated briefly in microwave oven to initiate silver salt reduction and then cooled to room temperature.

Separately, talc powder (0.5 g), IPA (1 ml) and water (4 ml) were mixed in a cup to get a uniform suspension. To the suspension 0.5 ml of the silver nanoparticles composition prepared above was added and mixed on a vortex mixer. The cream colored solids were recovered by centrifugation and drying in the oven at 45C for few hours.

Example 42 Aqueous silver nanoparticles containing composition

Sodium saccharinate (0.25 ml, 0.125M) and silver nitrate (0.25 ml, 0.1M) were added to water (1 ml) in a test tube. Tween 20 surfactant (0.05 g) was added to the resulting suspension followed by TEMED (0.05 ml) to start the reduction reaction. Within few minutes, yellow color appeared that intensified overnight. Absorbance of a diluted solution in water (dilution 1 to 5) was measured over 400 nm - 550 nm range. The maximum OD was observed at 415 nm.

Example 43 Aqueous silver nanoparticles containing composition

A composition with silver nanoparticles was prepared exactly as in example 42 except the volume of sodium saccharinate, silver nitrate and TEMED was doubled. The resulting solution showed a OD maximum at ~ 415 nm .

Example 44 Aqueous silver nanoparticles containing stock solution

In a cup, Tween 20 (0.5 g) was dissolved in water (10 ml). To this sodium saccharinate (10 ml, 0.125M), silver nitrate (10 ml, 0.1M) and TEMED (1 ml) were successively added. The liquid mixture was heated (30 seconds) briefly in microwave oven (Instamatic Cooking by Quasar) on MEDIUM setting. It turned yellow after heating due to the formation of silver nanoparticles.

Example 45 Polymer stabilized silver nanoparticles composition

Acrylamide (2.96 g) was dissolved in 25 ml of water. To the solution, ammonium persulfate (0.1 g) and TEMED (0.125 ml) were added, mixed to start polymerization. After 10 minutes, sodium saccharinate (1.25 ml, 1M) and silver nitrate (1 ml, 1M) were added to the viscous polymer solution. The solution color changed to orange red within minutes. The solution was warmed for 30 seconds in microwave oven if needed to speed up the reduction reaction. OD value peaked at a wavelength of 440 nm.

Example 46 Lubricating jelly

Lubricating jelly (BARD Inc., Covington, GA) with silver nanoparticles was prepared as follows. First, the nanoparticles solution was prepared and then blended with the jelly. CMC sodium salt (0.05 g, high viscosity grade, Sigma) was dissolved in water (10 mL). To the CMC solution (1ml), sodium saccharinate (1 ml, 0.125M), silver nitrate (1 ml, 0.1M) and TEMED (0.1 ml) were added in succession. The solution became yellow and imparted weak green fluorescence. To the lubricating jelly (8 g) in a cup, CMC-AgNP solution (0.2 ml) made above was added and mixed to uniformity with a glass rod. The jelly with silver nanoparticles imparted pale orange tint.

Example 47 Alginate beads

PAA-silver nanoparticles solution was prepared according to the method of example 40. The solution was added to sodium alginate solution (1 g/50 ml water). The resulting solution was added dropwise to a stirred 2% calcium chloride solution (400 ml) to form alginate beads embedded with silver nanoparticles. The beads were filtered and once washed with de-ionized water and stored wet. The beads imparted yellow color with trace green fluorescence.

Examples 48: Nail Polish Composition

A polymer used in nail polish application, Avalure 120 (1 ml) was mixed with silver nanoparticles solution (1ml) leftover from a preparation similar to Example A19 and spread over a clean glass slide and dried at 45° C. The dried film on the glass did not change color from

initial yellow even after more than two months indicating that there is no agglomeration of silver nanoparticles in dried films by diffusion mechanism.

Examples 49 Silver nanoparticles composition from potassium acesulfame

A composition comprising silver nanoparticles was prepared in a dram vial by mixing Tween 20 (0.3 ml, 65 g/L), potassium acesulfame solution (1 ml, 0.125 M), TEMED (0.3 mL) and lastly adding silver nitrate solution (0.75 mL, 0.1 M), vortexing after adding each ingredient. The resulting mixture was heated in microwave oven for 10 seconds, cooled and OD measured over 400 to 500 nm. The wave length maximum was found to be 415 nm.

Examples 50 Preparation of composition comprising silver nanoparticles from barbituric acid

Barbituric acid (0.368 g) was weighed and added to 10 mL deionized water. Sodium carbonate (0.105 g) was added to water to convert the acid to its sodium salt as the solution became clear.

Silver nitrate (1mL, 1M) solution was added to precipitate out silver barbiturate as fine suspension. To 1 mL silver salt suspension, 0.3 mL Tween 20 (65 g/L) and 0.3 mL TEMED were added and the mixture was heated for 10 seconds in microwave oven. A reddish orange color appeared indicating formation of silver nanoparticles. The wave length maximum was measured at 415 nm.

Examples 51 Silver nanoparticles composition from sodium saccharinate

A composition comprising silver nanoparticles was prepared in a beaker by mixing Tween 20 (1g) in 20 mL deionized water, then adding sodium saccharinate solution (20 ml, 0.125 mL), silver nitrate solution (20 mL, 0.1M) and finally TEMED (2.0 mL). The resulting mixture was heated in on a hot plate under stirring to 60-70° C over 15 min. Around 45C, the color change to yellow and continued to become darker. Some white precipitate was seen at the beaker bottom. The OD versus l curve measured over 400 to 500 nm was similar to a similarly made but microwaved solution. The wave length maximum was found to be 415 nm. The mode of heating did not alter the OD curve.

Examples 52 Non-aqueous silver nanoparticles composition from sodium oleate

An aqueous composition comprising silver nanoparticles was prepared in a test tube by mixing Tween 20 (0.3 mL, 65g/L), sodium oleate (1mL, 0.125M), TEMED (0.3 mL) and finally adding silver nitrate solution (0.75 mL, 0.1M) and heating it microwave oven briefly until the solution turned yellow. The OD maximum was observed at 415 nm. To the aqueous composition was added, toluene (2 to 3 mL) and vortexed to homogenize the contents that were left undisturbed for 2-3 weeks when all toluene had evaporated.

To the aqueous composition in the test tube, chloroform (3 mL) was added and shaken to extract the silver nanoparticles into non-aqueous chloroform layer. The chloroform layer turned amber brown as it gained copious amount of silver nanoparticles. The OD of the chloroform layer after dilution was measured over 300 to 550 nm. The maximum was seen at 420 nm and the shape of the curve was identical to the curve of the aqueous composition (see Figure 1). The aqueous liquid still rich with silver nanoparticles was re-extracted with a second portion of the chloroform (3 mL) to harvest more silver nanoparticles. A 1"x1" piece of a fabric woven from polypropylene having satin like finish was dipped in the 2nd chloroform layer and quickly removed and left to dry in air for few minutes. The fabric color changed from white to faint yellow/orange. In ZOI assay against Staphylococcus aureus it was found to be antimicrobial.

Examples 53 Silver nanoparticles composition from hydantoin

A composition comprising silver nanoparticles was prepared from hydantoin as follows: Silver hydantoinate was first prepared according to a method disclosed in example 2 of US Patent Application No. 2003/0186955. Next, silver hydantoinate (0.05g), deionized water (6.7 mL), Tween 20 solution (3 mL, 16.7 g/L) were mixed in a test tube and TEMED (0.3 mL) were added and contents vortexed and heated in microwave oven for 30 seconds to yield a yellow brown mixture. OD maximum of the mixture at 420 nm confirmed the presence of silver nanoparticles.

Examples 54 Non-aqueous silver nanoparticles composition

A non aqueous composition comprising silver nanoparticles was prepared as follows: Sodium oleate (3.3 mL, 4g/L) was used as stabilizer in place of Tween 20. It was mixed with sodium saccharinate (0.3 mL, 0.125M) in a test tube. To this mixture, silver nitrate (0.3 mL, 0.1M) was added followed by water (6 mL). Finally TEMED (0.17 mL) was added. The resulting mixture was microwaved for 20 seconds to warm it and initiate nanoparticles formation. Only faint color was observed. The contents now in a beaker were heated on a hot plate to evaporate all of the water. After most of the water was evaporated the beaker was cooled and 25 mL of chloroform added to extract silver nanoparticles. The chloroform imparted yellow color indicating the presence of silver nanoparticles. OD max was observed at ~ 430 nm.

Examples 55 Non-aqueous silver nanoparticles composition

A non aqueous composition comprising silver nanoparticles was prepared as follows. First an aqueous composition comprising silver nanoparticles made in proportions similar to in Example 44 and allowed to evaporate to a viscous brown mass. To this mass chloroform (2-3 mL) was added to extract silver nanoparticles. At once the chloroform layer became yellow

brown. OD max was 415 nm and in shape the OD vs wavelength curve was similar to that in example 52. Few drops of chloroform layer obtained were spread on a glass slide. Upon drying the film gave shiny appearance and imparted turquoise color.

Example 56 Aqueous silver nanoparticles compositions with CMC as stabilizing agent

CMC Na salt solution was prepared by dissolving 0.05g polymer in water (10 mL). In a test tube, CMC solution above (1 mL), sodium saccharinate (1 mL, 0.125M) and silver nitrate (1 mL, 0.1M) were mixed. Finally, TEMED (0.1 mL) was added and mixture vortexed. Yellow color change to the solution was observed within few minutes indicating nanoparticles formation. The solution color intensity increased with time. The solution also imparted green fluorescence. OD max was observed at 438 nm.

Example 57: Aqueous silver nanoparticles compositions with CMC as stabilizing agent

In the example 56 above, the sodium saccharinate was replaced with potassium acesulfame salt solution and preparation repeated. Again yellow brown color due to silver nanoparticles in solution was observed. OD was not recorded. The preparation was repeated with potassium acesulfame salt instead of sodium saccharinate. The solution obtained once again imparted yellow brown color indicating the presence of silver nanoparticles.

Example 58 Aqueous silver nanoparticles compositions with Propylene glycol alginate as stabilizing agent

In the example 56 above, the CMC Na salt was replaced by propylene glycol alginate and preparation repeated. OD maximum was found to be 440 nm. The solution also imparted green fluorescence but less in intensity that in Example 56.

Example 59 Aqueous silver nanoparticles compositions using various surfactants as stabilizing agents

Surfactant stock solutions were made at ~ 65 g/L using Tween 20, Tween 80 and Polyoxyethylene stearate. To prepare silver nanoparticles comprising solutions, a given surfactant solution (0.3 mL), acesulfame potassium salt solution (1 mL, 0.125M), silver nitrate solution (0.75 mL, 0.1M) were mixed and then TEMED (0.3 mL) were added. The solutions were heated in microwave oven briefly until the solution became yellow. OD versus wavelength data was recorded for each surfactant (Figure 18). Though small different in the maxima was seen all were in 415-425 nm range indicating consistent nanoparticles size.

Example 60 Silver nanoparticles compositions prepared using triethanolamine

Silver saccharinate powder was prepared from equimolar mixtures of silver nitrate and sodium saccharinate solutions. Silver saccharinate powder (30-35 mg) was added to

Tween 20 solution (1 mL, 16.7 g/L) and then water (4 mL) was added. To this mixture, triethanolamine (0.225 g) was added and it was briefly heated in microwave until the content became yellow.

Various articles with antimicrobial property were prepared using this above composition. Nylon fibers were made by dipping for 2 hours at 55° C and rinsing them. Cotton gauze and satin pieces (2"x2") were prepared by dipping them in the above composition for a minute, then blotting them and soaking them in ethanol (10 mL) for 5 minutes, re-blotting them and drying at 55° C for 15 minutes.

Example 61 Silver nanoparticles compositions prepared using poly vinyl alcohol (PVA)

PVA solution was prepared in de-ionized water (0.02-00.03 g/10 mL). PVA solution (1 mL), sodium saccharinate (1 mL, 0.125M) and silver nitrate (1 mL, 0.1M) were vortex together. TEMED (0.1 mL) was added and vortexed again. The contents were briefly heated in microwave oven. The solution turned grey brown though theOD max of the solution was 455 nm.

Example 62 Silver nanoparticles compositions using polyacrylamide (PAA) as stabilizer

An identical test to Example 61 was carried out but instead of PVA, poly acrylamide was used. PAA was made as a concentrate and 0.05 g concentrate was added to 1 mL water. The OD maximum of the composition was 450 nm and its color was brown.

Example 63 Silver nanoparticles compositions using polyvinyl pyrrolidone (PVP) as stabilizer

In Example 61, PVP was replaced with PVP solution (0.25 g/10 mL water) and the test repeated. The resulting composition after heating turned green instead of yellow. The OD max was seen at 435 nm with the spectrum being less sharp than in the case of use of Tween 20 indicating a broad particle distribution.

Example 63 Silver nanoparticles compositions using potassium sorbate as stabilizer

A solution of potassium sorbate (0.1M) was prepared. The sorbate solution (1 mL) was mixed with Tween 20 (1 mL, 16.7 g/L), and silver nitrate (1 mL, 0.1M) were vortex together. TEMED (0.05 mL) was further added and vortexed again. The contents in a test tube were briefly heated when solution color changed to orange yellow. The composition OD maximum was 410 nm. This example shows that one can use a double bond containing molecule (silver sorbate) as the source of silver.

Example 64 Silver nanoparticles composition using Sodium Oleate w/o Tween 20

Sodium oleate (4-5 mg) was dissolved in 1 ml water in a test tube. To which were added sodium saccharinate (1 mL, 0.105M) and silver nitrate (1 mL, 0.1M) to give a chuncky white

precipitate. To the test tube TEMED (0.2 mL) was added and briefly microwaved to heat the contents. Upon heating a color change to yellow took place indicating formation of silver nanoparticles. OD of the maximum was not recorded.

Example 66 Silver composition comprising silver-TEMED complex

Tween 20 solution (1 mL, 16.7 g/L) and silver nitrate (1 mL, 0.01M) were mixed in a test tube. Then TEMED (0.1 mL) was added to briefly heat in microwave oven to deposit silver as metallic film on tube walls. The area of the glass surface coated with purplish metallic film became poorly water wetting as indicated by the flat water-air interface instead of a curved interface.

Example 67 Silver composition comprising sorbate –Effect of ethanol on stability

Solutions of silver nanoparticles composition of Example B27 were prepared by diluting with water and 66% water-33% ethanol mixture (1: 100 dilution factor). The UV/VIS scans were recorded of either solution fresh and of the water-ethanol based solution after 5 days. No change in the spectra was observed indicating tolerance of silver nanoparticles to ethanol.

Example 68 Use of different amines as reducing agents

Tween 20 solution (1 mL, 16.7 g/L), sodium saccharinate (1 mL, 0.125M) and silver nitrate (1 mL, 0.1M) were vortexed together. Different amines (0.1 mL) was added and vortexed again. If needed, the contents were briefly heated in microwave oven. The OD maxima of the solutions were recorded.

Following amines were tested: N,N, N'N' tetramethyl butylenediamine, ethanolamine, cyccohexylamine, dipropylamine, triethanolamine. Of these dipropylamine and triethanolamine successfully gave yellow colored solution indicating the presence of silver nanoparticles with identical solutions OD maxima at 415 nm and practically identical spectral shapes of the curves.

Example 69 Silver composition using powder form of silver saccharinate

Silver saccharinate powder (15-20 mg) was added to Tween 20 solution (1 mL, 16.7 g/L) and then water (2 mL) was added. To this mixture, triethanolamine (0.1 g) was added and it was briefly heated in microwave until the content became yellow. The OD max of the solution was 420 nm and the shape of UV-VIS spectrum was identical to a composition made by in-situ formation of silver saccharinate.

Nylon fibers were made by dipping in silver nanoparticles composition above for 2 hours at 55° C and rinsing them. Cotton gauze and satin pieces (2"x2") were prepared by dipping them in the above composition for a minute, then blotting them and soaking them in ethanol (10

mL) for 5 minutes, re-blotting them and drying at 55° C for 15 minutes. The fibers exhibited antimicrobial activity.

Example 70: Silver composition comprising fumarate

Sodium fumarate was made as follows: 0.116 g of fumaric acid was added to 10 ml water in a test tube. Further, 2 molar equivalents of sodium carbonate were added to form sodium fumarate. Without isolating sodium fumarate, 1 ml of the sodium fumarate solution above, Tween 20 solution (1 mL, 16.7 g/L) and silver nitrate (1 mL, 0.1M) were mixed in succession and then TEMED (0.1 mL) was added. The tube contents were heated briefly in microwave to yield a yellow colored solution with OD max of 420 nm. Without Tween 20, the solution color is purple..

Example 71: Silver nanoparticles comprising gel

In a cup, glycerol (5.0 g) was weighed, carboxymethyl cellulose (0.5 g) was added and hand mixed to coat cellulose particles uniformly with glycerol. Warm de-ionized water (40 mL) was added to the cup and the resulting mass mixed to yield smooth gel. Silver nanoparticle composition made from triethanolamine (0.1 g) from example 60 was added and mixed to uniformity to give a yellow colored gel. To a portion of the gel (10 g), 1 g each of citric acid and water were added to provide an antimicrobial gel that could be used in the treatment of onychomycosis.

Example 72 Silicone based conductive elastomer

Twelve silicone test strips (Type BMSI-7Z or 72B, Meggitt Silicone Products, McMinville, OR) in the shape of a dog bone (4.5" long and " wide at ends, 2.5" long and 0.25" wide in the neck) were immersed in 99% isopropanol in a glass beaker and sonicated for 10 minutes (Fisher Scientific Sonicator Model FS30), excess liquid rinsed off, and dried in an oven at 45° C for 10-15 minutes. The test strips were then transferred to a container with 450 ml 23% nitric acid and slowly shaked overnight (or for 24 hours) at 25° C on a see saw rocker. The strips were thoroughly rinsed with deionized water until there was no trace of acid in the rinse water. In another container silver nanoparticles solution was prepared by mixing Tween 20 solution (160 ml, 16.7 g/L), sodium saccharinate solution (160 mL, 0.025M), and silver nitrate solution (160 ml, 0.1M). The mixture was stirred for 5 minutes after each solution addition.

The solution was heated in microwave oven briefly and the heating was stopped when the solution temperature reached ~ 55° C. In a shallow Pyrex dish the strips were laid flat on a nylon screen and the hot silver nanoparticles solution was poured over the strips to immerse the strips completely. The strips were left in the oven at 55° C for 18 h. The treatment with silver

nanoparticles was repeated twice but the duration was increased to 24 h. Prior to the second silver treatment, the test strips were washed first with 200 ml Tween 20 solution (4.2 g/L). After the second silver treatment, the strips were rinsed once again with Tween 20 solution (4.2 g/L) followed by tap water rinses and then dried in the oven at 45° C for 15-20 minutes. Four strips were removed and saved for another experiment. A third silver treatment was carried out on the remaining 8 strips using a silver nanoparticles solution made from 100 mL each of Tween20 solution (16.7 g/L), sodium saccharinate solution (0.025M), and silver nitrate solution (0.65M) for 16 h at 55° C.

Following the third silver treatment, the strips were rinsed with deionized water, sonicated in water and in isopropanol for 10 minutes each and then left on paper towels to air dry. Each piece imparted a greenish turquoise metallic shine that was fairly uniform. When probed with a multi-meter (Extech Instruments), no electrical continuity was observed on any of the strips. Each strip was then flame annealed by passing the strip across over butane flame from a Lenk butane flame burner (Model 65) several times. Care was taken not to cause any burning of underlying silicone. The strips were then cooled to room temperature and tested for electrical continuity under zero strain and a maximum of ~ 300% strain. Electrical resistances in the ranges of 3 to 20 ohms were recorded when probed across the strip length. When strained to 300%, resistance values of 1 to 3 kiloohms were recorded. Not all 8 samples showed continuity at 300% strain but all showed continuity up to varying degrees of strain. Even after multiple strain cycles, electrical continuity was not lost in the test samples suggesting robustness of the deposited silver layer. Despite deposition of silver layer on the strips, increase in their weight post silver treatment was negligible indicating very thin layer of silver was deposited.

Example 73 Silicone based conductive elastomer

The extra 4 test strips prepared in the Example 72 were treated slightly differently. The strips were treated with Tollens reagent to deposit silver at a much faster rate than the rate in the third treatment in Example 72. The test strips were dipped for 10 sec in a solution made by dissolving stannous chloride ($SnCl_2.2H_2O$, 2.5 gm) in 50 ml deionized water and 5 ml concentrated HCl, then rinsed with water and air dried briefly. Next the strips were immersed for 6 mins in Tollens reagent solution at 25 °C made by mixing silver nitrate (0.1M, 196 mL), sodium hydroxide (10%, 16 mL), ammonium hydroxide (25%, 112 mL) and glucose solution (0.1M, 48 mL). The strips were removed, rinsed with water, air dried and flame annealed as in Example C1 over butane flame. When tested for electrical continuity under strain, they registered electrical resistances higher than those samples in Example 72.

Example 74 Silver based flexible mirror

A flexible mirror was also constructed by the inventors. A Kapton® polyimide adhesive tape (about 3' long and 0.5" wide) was applied to a clean glass slide. The glass slide was suspended from a hook such that the tape was completely immersed in ~ 150 mL silver nanoparticles solution in a cup maintained at 55° C for 4 h. The solution was prepared by mixing 50 mL each of Tween 20 solution (5.6 g/L), sodium saccharinate (0.025M), silver nitrate (0.1M), and TEMED (5 mL). It was heated to 55° C in a microwave oven.

After silver treatment, the slide and the film was thoroughly rinsed with water, sonicated in water for 10 minutes to remove loose debris, dried with hot air gun. The polyimide was deposited with a shiny reflecting mirror of silver. Half of the mirror was flamed annealed as described in Example 72. The annealed portion was found to adhere better to the underlying Kapton® tape whereas the non-annealed region could be rubbed off. The annealed portion could be bent without the silver mirror flaking off indicating good adhesion.

Example 75 Kapton film with silver coating

A Kapton strip was coated with nanosilver using as in Example 74 above but was treated for 1h at 55° C instead. The resulting shiny reflective Kapton strip was taped to a glass slide with Scotchgard® tape to keep it flat during annealing. The silver coating was annealed by butane flame by running it lengthwise (~ 1 min with pauses to cool the strip). The cooled film was examined for conductivity by measuring its resistance lengthwise (~ a distance of 7-8 cm). A resistance ranging 100 to 3000 ohms was observed at several points, showing the silver coating became conductive after annealing. The strip was wrapped around the 2 mm thick glass slide and still read resistance values observed before. The annealed silver coating showed bend resistance.

Example 76 Coated Acrylic sheet

A coated Acrylic polymer (supplied by Rohm & Haas Co., Philadelphia, PA) strip1cm wide and ~ 8 cm long was immersed in a solution identical to that in Example 74. After 1h at 55° C, the strip was removed, rinsed with Tween 20 solution (4.3 gm/L) and de-ionized water. The strip was treated at 55° C for 1h second time using freshly made identical silver containing solution to deposit more silver. The sample was flame annealed as in Example 2, cooled and tested for electrical continuity. Lengthwise over 7-8 cm the silver coating was conductive with resistance values measuring 30 – 34 kiloohms.

Example 77 Electrically conductive tulle material

Tulle material made of polyamide polymer (purchased from a local fabric store) in the form of 2"x2" squares (total- 10 samples) were immersed in a solution made from 200 mL Tween 20 (16.7 gm/L), 200 mL 0.075M sodium acetate and 200 mL silver nitrate (0.1M) and TEMED (20 mL). The solution was heated to 55° C and after 2, 4, 6, 9 and 12h period two samples each were removed rinsed with 10% ammonium hydroxide solution and then with de-ionized water and dried. The samples treated for 6h or more showed a metallic shine with a purplish tint. The metallic silver coating was uniform on the nylon thread making up the tulle material.

Using banana clips on the nylon thread at the diagonal corners of the samples, the electrical resistance of the samples was measured. The samples treated for 6h or more –all exhibited resistance values in the range 5 – 15 ohms clearing showing them to electrically conductive. Even after wrapping the sample piece around sharp bend did not change the resistance readings. Even sonication of pieces for 10 minutes immersed in water did not alter the resistance values indicating extraordinary adhesion of the resulting nanoparticle silver coating. None of the samples treated for 6h or more required annealing for the silver coating to be electrically conductive.

Example 78 Electrically conductive fluorosilicone elastomer

A strip 1"wide and 3" long made of fluorosilicone elastomer were supplied by Meggitt Silicone Products of McMinnville, OR. The strip was wiped with isopropanol and air dried. The strip was deposited with nanosilver in 3 steps. In step 1, a solution was prepared by mixing 40 mL Tween 20 (16.7 gm/L), 20 mL 0.125M sodium saccharin, 20 mL 0.125M sodium acetate and 40 mL 0.15M silver nitrate solutions. To this solution, 12 gm triethanolamine (TEA) was added to yield a clear solution. After heating the solution in microwave oven to ~ 55° C, the elastomer strip was immersed in it. The contents were maintained at 55° C for 21h, then removed and rinsed with de-ionized water. Next, step 2 was carried out which was a repeat of Step 1. The silver coated strip was removed again and rinsed thoroughly with water. It was cut into two identical pieces (1"x1.5"). One piece was annealed on butane flame and tested for electrical continuity. On multi-meter display (Extech Model MiniTec 26™) we did not get a measurable reading indicating the coating to be insulating. The remaining piece was subjected to Step 3. The sample was immersed in a solution made with 40 mL each of Tween 20 (16.7 gm/L), 0.025M sodium saccharin and 0.25M silver nitrate solutions. Triethanolamine (2 gm) was also added. The tub bearing solution and the sample (spaced from the tub bottom by a nylon

screen mesh piece) were kept at 55° C for 24h. After the treatment, the sample was rinsed thoroughly – first with Tween 20 (4.3 gm/L), tap water and finally with de-ionized water. The initially sky blue colored sample imparted silver ash color with matte finish. When handled, the silver tended to flake off slightly. Before annealing, we recorded resistance values of the silver coated fluorosilicone elastomer. One surface of the sample, the values when measured with probe leads diagonically across were between 2 and 5 ohms (the side away from tub bottom) and the surface closer to the bottom had higher values (200 to 500 ohms). The difference we surmise is due to different rates of silver deposition on the top versus bottom surfaces. Annealing the sample piece, did not alter the resistance values very much, but the sample surface became silver grey with increase in metallic shine. The conductivity of the fluorosilicone sample had resistance values < 5 ohms.

Example 79 6"x6" silicone elastomer slabs

A total of 50 6"x6" silicone elastomer slabs were coated with silver. To prepare the slabs for coating, they were threaded with a strong fish line through two points each spaced 1" from the top and respective side edges of the slab. This allowed the slab to be suspended without touching the bottom of a Sterilite® 1 gallon polypropylene pitcher. 17 slabs were treated in two separate pitchers with the third holding 16 slabs. The slabs suspended inside pitcher were rinsed with Tween 20 solution (4.3 gm/L) and then de-ionized water. Excess liquid was drained off from the pitchers and the slabs were treated with silver solution without further drying as follows.

The slabs were treated to three different levels of silver loading – low, medium and high. Each level was to achieve different level of conductivity (or resistance). Following steps were involved in producing slabs with silver coating.

Stage 1 @ 55° C for 24h

Treatment of the slabs with a solution made from1 volume part Tween 20 (16.7 gm/L);1 volume part 0.025 M sodium saccharin;1 volume part 0.1 M silver nitrate, and 0.1 volume part TEMED (tetramethyl ethylene diamine). The slabs were rinsed with tap water and once with de-ionized water.

Stage 2 @ 55° C for 18h

Treatment of the slabs with a solution made from1 volume part Tween 20 (16.7 gm/L), 1 volume part 0.025 M sodium saccharin, 1 volume part 0.1 M silver nitrate, and 0.1 volume part TEMED. The slabs were rinsed with tap water and once with de-ionized water.

Stage 3 @ 55oC for 24h

Treatment of the slabs with a solution made from1 volume part Tween 20 (16.7 gm/L), 1 volume part 0.025 M sodium saccharin, 1 volume part 0.25 M silver nitrate, 0.1 volume part TEMED The slabs were rinsed with tap water and once with de-ionized water (and air dry if low level slabs were made)

Stage 4 @ 55° C for 4h

Treatment of the slabs with a solution made from 1 volume part Tween 20 (16.7 gm/L),1 volume part 0.025 M sodium saccharin, 1 volume part 0.25 M silver nitrate, and 0.1 volume part TEMED. The slabs were rinsed with tap water and once with de-ionized water and air dried at room temperature

Stage 5 @ 55° C for 16h

Treatment of the slabs with a solution made from 1 volume part Tween 20 (16.7 gm/L), 1 volume part 0.025 M sodium saccharin, 1 volume part 0.25 M silver nitrate, and 0.1 volume part TEMED. The slabs were rinsed with tap water and once with de-ionized water and air dry at room temperature

To produce slabs, the following protocols were followed: Low level – Stages 1 to 3; Medium level – Stages 1 to 4; and High level – Stages 1 to 3 and 5.

Finally, all slabs were flame annealed ~ 15's on each side on a propane heater and cooled to room temperature. The resistance values were measured across the two diagonals on each side and presented in tables below. The values typically are in megaohms for low loading slabs; are of the order of kiloohms for medium loading and are in tens of ohms for high loading. The gradual decrease in resistance values indicated that the treatment variation was achieving the desired goal of having different thicknesses of silver coating. Random measurements of resistance values with some slabs samples under bending strain showed electrical continuity and registering only very small increase.

Table 15: Resistance Values in mega ohms of Silver Coated Silicone Slabs (Low Loading)

	Side 1		Side 2	
Sample No.	Diagonal 1	Diagonal 2	Diagonal 3	Diagonal 4
1	2.4	1.9	1.85e-4	5.3e-5
2	15.0	3.0	5.0	9.0
3	1.15e-4	3e-4	9.8e-5	1.3e-3
4	1.27e-4	1.53e-4	8.4	2.3e-3
5	8e-4	1.38e-4	2e-3	1.1e-3
6	12.5	10.0	22.0	5.5e-2

Table 16: Resistance Values in ohms of Silver Coated Silicone Slabs (Medium Loading)

	Side 1		Side 2	
Sample No.	Diagonal 1	Diagonal 2	Diagonal 3	Diagonal 4
1	23	25	53	55

2	430	460	480	750
3	165	147	26	53
4	75	69	14	80
5	2500	242	100	89
6	8	13	16	14

Table 17: Resistance Values in ohms of Silver Coated Silicone Slabs (High Loading)

	Side 1		Side 2	
Sample No.	Diagonal 1	Diagonal 2	Diagonal 3	Diagonal 4
1	7	8	12	5
2	3	16	124	32
3	5	4	7	9
4	30	20	90	46
5	8	9	9	8
6	30	22	18	7

Example 80 Silver coated fluorosilicone elastomer

Three strips (1"x3") of fluorosilicone similar to the one used in example 79 were coated at low, medium and high levels of silver following the method in example 80 except instead of TEMED triethanolamine was used. This yielded one strip at low, medium and high silver loading. The strips were annealed as in example 8 and examined for electrical conductivity using multi-meter. We observed no measurable resistance values for low and medium coated samples but the high level samples showed reading in the range of 20 -30 megaohms.

To determine the amount of silver coated, we cut thin slivers from the coated pieces and stripped them of silver by treating them with a mixture of 30% Hydrogen peroxide and concentration nitric acid. The solutions with dissolved silver were analyzed for silver by FAAS. The amount of silver at low, medium and high loading were found to be 0.33 mg/cm2, 0.8 mg/cm2 and 1.35 mg/cm2 respectively.

Example 82 Silver coated silicone elastomer

Silicone elastomer in the shape of a dog bone (~ 3.5"x1.0"x0.063" with 0.25"wide and 1.5" long stem in the center) was soaked in 23% nitric acid overnight and rinsed with de-ionized water and dried. It was treated with silver nanoparticles solution made by mixing equal volumes of Tween 20 (16.7 g/L, 70 mL), sodium saccharinate (0.025M) and silver nitrate (0.1M) followed by TEMED addition (7 mL). The mixture was warmed to 55° C in a microwave oven upon which it turned clear dark brown. The dog bone was immersed in solution for 17h at 55° C, rinsed with water and dried. The initial light gray piece turned light grey black in color after silver treatment.

It was re-treated using a fresh silver solution made the same way for 24h at 55° C. Next, it was rinsed and sonicated in water for 10 minutes at 25° C. The initial light gray piece now looked more silvery in color after 2nd silver treatment.

A sensitizing solution was prepared by dissolving 0.5g SnCl2.2H20 and 0.5g concentrated HCl in 10 mL de-ionized water. To this solution, were added isopropanol (5 mL), concentrated HCl (4 mL) and de-ionized water (31 mL). The silver coated dog bone was immersed in the sensitizing mixture for 10-15s, rinsed thoroughly with water and dried with a hot air gun. Yet another silver containing solution (Tollen's reagent) was prepared as follows. To silver nitrate solution (0.1M, 48 mL), sodium hydroxide solution (10% w/v, 4 mL) was added to yield a brown precipitate. To the precipitate was added ammonium hydroxide (7% v/v) in just enough volume to get a clear colorless solution. To this silver-ammonia complex solution, freshly made glucose solution (0.1M, 12 mL) was added. Half amount of the total volume of the solution prepared was transferred to a 50 mL polypropylene tube and the dog bone strip immersed in it for 6 min at 25° C. The strip was removed and rinsed with water and air dried. The strip thus obtained was flame annealed over butane flame. The dog bone strip was found to be electrically conductive (resistance < 5 ohms). Under stretching to ~ 300% the resistance value observed was in < 10 kiloohms. The strip continued to show conductivity (R< 5 ohms) even after repeated strain fatigue cycles. Even after 3-4 twists, the resistance measured lengthwise was < 50 ohms. Even after 1 year, the same type of electrical conductivity behavior in the sample was observed. The amount of silver deposited on the dog bone was determined to be ~ 2.9 mg/cm2.

Example 82 Golf ball – Method 1

A golf ball was treated to deposit silver on its surface. An aqueous mixture was made by mixing equal volumes (30 mL) of Tween 20 (16.7 gm/L), sodium saccharinate (0.1M) and silver nitrate (0.1M). To the milky suspension, TEMED (3 mL) was added under gentle stirring. The content were transferred to a 125 mL capacity glass container containing the golf ball. Enough solution was poured to keep the ball submerged. The container was capped with a lid and placed in an oven at 55° C for 24h. The treatment was repeated to yield a golf ball surface impregnated with silver. After the repeat treatment, the ball was rinsed thoroughly with deionized water and left to air dry. The ball had a yellow brown color that was very uniform.

Example 83 Golf ball – Method 2

A golf ball was treated to deposit silver. The silver containing solution was prepared by mixing ~ 67 mL each of Tween 20 (16.7 gm/L), sodium saccharinate (0.075M) and silver nitrate

followed by TEMED (6.7 mL). The solution was heated in microwave to ~ 60° C and poured over the golf ball, which was placed in glass beaker, ensuring the ball remained submerged. The beaker was covered to prevent the liquid from evaporating. The beaker was placed in an oven set to 60° C for ~ 20h. The ball was removed and rinsed with water and left to air dry. The color of the ball turned yellow from the initial white the intensity of which increased with time. In feel the golf ball was no different than before. During the treatment, there was no loss of the logo image. Though not tested, the silver coated ball is expected to be bacteriostatic.

Example 84 Polycarbonate Film

A ~ 8 cm x 1cm (~ 0.1mm thick) film strip made of polycarbonate was cut from a sheet and transferred to a polystyrene tube with a cap. Separately, a silver containing mixture was prepared in a glass test tube by mixing 2 mL each of Tween 20 (16.7 gm/L), sodium saccharinate (0.075M) and silver nitrate (0.1M) and TEMED (0.2 mL) in that order. The mixture was heated in microwave oven to ~ 55° C when its color became light yellow. The hot mixture was transferred to PS tube containing sample strip and the tube heated for 16h at 55° C in an oven. After the treatment, the sample was washed with water and air dried. The portion of the strip that was immersed in solution had turned uniformly amber colored. Iridescent shades of magenta, blue and metal were observed on the strip surface. The amber color indicated presence of silver nanoparticles on the surface.

Example 85: Polycarbonate Film

A strip was prepared exactly as described in example 85. The strip was sonicated for 10 minutes to remove loosely adhering particles. The amber colored portion of the strip was treated a mixture made with Tween 20 (16.7 g/L, 3 mL), silver nitrate (16.7 g/L, 3 mL) and TEMED (0.3 mL) for 1h at 55° C. The strip color was much darker than before and its surface somewhat shiny. The darker amber shade than before suggested the strip gained more silver than in the sample in example 85.

Example 86 Polystyrene Substrates

This example describes the method of silver deposition on various polystyrene based articles. For illustration tubes and well plates were used, but the method described is applicable to all types of polystyrene articles and surfaces. A polystyrene tube was uniformly coated on the inside with silver. The coated surface had metallic shine and in ambient lab light, the color of the coated layer was reddish brown.

Example 87 Glass Substrates –Formation of a silver mirror

This example illustrates the use of glass slide but the method is applicable to other articles made from glass. In a petri-dish, a piece of nylon mesh was placed as spacer between the dish surface and glass surface. A solution made from Tween 20 (16.7 g/L, 20 mL) and silver nitrate (0.1M, 20 mL) and TEMED (2 mL) was poured over the slide. The petri-dish was sealed and place in an oven at 55° C for 1-2h. The solution was discarded and glass slide was thoroughly was with de-ionized water and dried with a heat gun. A very reflective shiny silver mirror was obtained. When held to light, the silver mirror imparted purple blue color and was transparent. The mirror was electrically conductive registering a resistance value of 55-65 ohms along the length of the glass slide.

Example 88 Polyurethane tubing stock

This example describes a method to deposit silver on polyurethane class of materials. Though the illustrative example uses tubing stock, the method is applicable to all polyurethane based articles. Polyurethane tubing stock was cut in 30 inches long sections. 6 tube segments were wrapped around a rod and zip-tied. The tube segments were placed inside a tubular reactor containing a silver solution made from equal volumes of Tween 20 (16.7 g/L, 120 mL), sodium saccharinate (0.075M) and silver nitrate (0.1M). The contents were heated to 55° C under gentle rocking (~ 10- 12 oscillations/min). After 55° C was reached the reactor was opened briefly and TEMED (12 mL) was introduced and reactor lid closed. The contents were maintained at 55° C for 3h. The reactor was opened and the spent silver solution was drained. Then 600 mL of 1:4 diluted ammonium hydroxide was poured and the reactor rocked for 15 minutes. After draining ammonium hydroxide, the samples were removed and washed thoroughly with lots of de-ionized water, centrifuged to remove water inside lumen and left to air drying on clean paper overnight. After silver treatment, the catheter segments imparted yellow brown color. Silver analysis by FAAS showed a loading in the range 7-10 μg/cm2.

Example 89 Glass Prism

A glass prism (sides ~1.5" and height ~ 1.0" and ~ 0.5" deep) was washed in a sonicator (Fisher Scientific Model FS 30) for 5 minutes each in, 10% nitric acid, 10% sodium hydroxide solution, isopropanol and 1:10 diluted ammonium hydroxide in succession and then placed directly in a solution made by mixing Tween 20 (16.7 g/L, 50 mL), silver nitrate (0.15M, 50 mL) and TEMED (5 mL) and heated to 55° C for 18h. After treatment was complete, the prism was removed and washed with Tween 20 solution (16.7 g/L) followed by thorough rinsing with de-ionized water. The measured reflectance of silverized glass prism was 88-90 % for > 500 nm.

Example 90 Titanium disks

This example describes silver nanoparticle deposition on titanium disks (32 mm dia and 2 mm thick). The method is applicable to titanium substrates of all kinds with minor variations as needed. Twenty disks were placed in a warm solution (55° C) obtained after heating a mixture of 1.3 liters each of Tween 20 (16.7/g/L), sodium acetate (0.075M) and silver nitrate (0.15M) and TEMED (0.13 liter). The tub holding the solution and disk was capped and placed on a shaker in an oven set at 55° C for 18h. The tub was removed, liquid drained off and the disks quickly placed in another container with 500 mL of wash solution (10% v/v ammonium hydroxide) for 1 minute and then washed with de-ionized water, patted dry with tissue paper and air dried. After the treatment, there was very little visible difference. The silver loading was estimated to be ~ 20 μg/cm2.

Example 91 Gold screws

This example describes deposition of silver nanoparticles on gold surfaces. For illustration gold screws that are commonly used in dental medicine were used. Fifty screws with gold surface (~ 0.35" long, ~ 0.08" dia, 0.075" screw head dia and 0.15" threaded length) were treated with silver containing solution at 55° C for 16h. The treating solution was made from Tween 20 (16.7 g/L, 125 mL), sodium saccharinate (0.125M, 75 mL), silver nitrate (0.1M, 50 mL), de-ionized water (125 mL) and TEMED (12.5 mL). The screws were sealed in a nylon mesh satchel to prevent their accidental loss and to expedite cleaning. After the treatment, the satchel with screws was immersed in a beaker filled with de-ionized water and rinsed thoroughly, and the screws were then left to air dry on paper. Deposition of silver on the gold screws was evident from gold surface turning silver white in color. The amount of silver deposited was determined by FAAS as ~ 24 μg/cm2.

Example 92 Copper substrates

This example demonstrates the deposition of silver nanoparticles on copper articles. For illustration, a US copper penny coin was used. The method can be applied to all copper surfaces. A solution of silver was prepared by mixing Tween 20 (16.7 g/L, 4 mL), sodium saccharinate (0.075M, 4 mL), silver nitrate (0.1M, 4 mL) and TEMED (0.4 mL) in that order. The solution in a 50 mL PP tube (Falcon Brand) was heated to 55° C in microwave oven, cooled to room temperature and then poured over a clean copper penny (bright colored) placed over a mesh in a Petri dish. The penny was kept submerged in the liquid overnight. It was rinsed, sonicated for 3 minutes in water, wiped dry gently to yield silver coated ash grey penny.

Example 93 Silicone tubing stock

This example describes deposition of silver nanoparticles on clear silicone tubing stock (OD: 3.1 mm and ID: 1.5 mm) that is commonly used in urinary catheters. While the exemplary substrate is tubing, the method of treatment can also be readily applied to silicone based articles. A silver containing solution was prepared by mixing 20 mL each of Tween 20 (16.7 g/L), sodium acetate (0.05M) and silver nitrate (0.15M) followed by TEMED (2 mL). To 50 mL capacity polypropylene (PP) tube (BD Falcon brand), 10 pieces of 1 cm long pieces of tubing were added and then 10.33 mL of the silver solution pipetted. Three PP tubes in total were prepared and placed on a shaker inside an oven at 55° C. One tube was removed after 2h, the 2nd tube after 3h and the 3rd tube after 4h. Each time, the sample pieces were poured in Tween 20 solution (4.2 g/L, 50 mL), then rinsed with de-ionized water and left to dry in air overnight. The clear tubing pieces became yellow brown to dark brown with increased treatment time. The silver loading on tubing stock treated for 2, 3 and 4h was determined as 8.4, 11.1 and 13.4 μg/cm2 respectively.

Example 94 Luer activated device composed on Polycarbonate and Silicone

This example describes the method of depositing silver nanoparticles on polycarbonate and silicone surfaces of a Luer activated device. The medical device consists of three parts – polycarbonate based housing and base and a silicone gland allows for needleless connection for introducing fluids into the human body. While the device treated was chosen as illustration, the treatments can be applied to any polycarbonate or silicone based articles.

The housing was treated as follows. 2500 housing pieces were placed a basket in a tank lined with polypropylene liner. A silver containing solution was made using 5 liters each of Tween 20 (16.7 g/L), sodium acetate (0.05M) and silver nitrate (0.15M). To this solution TEMED (0.5 liter) was added. The tank was heated to 55° C and the heating maintained for 24h. The pieces were removed, rinsed with Tween 20 (4.2g/L), 10% ammonium hydroxide and de-ionized water and allowed to air dry. Of the treated pieces, 400 were treated second time with the same silver solution for 1h at 55° C maintaining the solution volume per piece to ~ 6 ml.

The base was treated identically to housing as described in the preceding paragraph. Both pieces turned shiny grey black after treatment. Similarly, the silicone gland was treated using same chemical recipe at 55° C but the treatment lasted ~ 9.5h without the need for a re-treatment. The fluid volume to part ratio remained the same. The silicone piece was turned greenish grey with shiny but non-reflective surface.

The silver coated components were assembled into luer activated devices and sterilized by gamma irradiation before use in biofilm assay. The amount of silver on the housing base and gland was estimated by FAAS as ~ 36.6, 25.6 and 93.0 μg/cm2.

Example 95 Biofilm Fouling Assay

Surfaces exposed to fresh and marine water over time will foul i.e. form a slippery layer due to the formation of biofilm. It is known free floating i.e. planktonic microorganisms generally will not adhere to surfaces however some microorganisms develop an ability to form polysaccharide film i.e. convert to biofilm forming counterparts after they have adhered to surfaces. They colonize this layer and continue to build and ultimately spread the film all over the surface. Fouled surfaces may affect hydrodynamics – increase resistance to flow and heat and may affect the aesthetics of water conveyances such as boats.

The present invention eliminates the problem of biofilm formation by deposition of nanoparticles, such as silver nanoparticles, on surfaces. The assay was applied to evaluate silver coated polycarbonate and silicone surfaces of a luer activated device. However, its application to these surfaces is for illustration and not to be construed as limiting. To the contrary the assay with minor variations can be applied to assess biofilm inhibition by nanoparticle coating on different polymers, metals and ceramics. The principle of the assay involves allowing microorganisms to form and grow biofilm of the surface and then evaluate biofilm formation by sloughing off biofilm from the surface using sonication and plating the sloughed off biofilm containing fluid to enumerate surviving bacteria. Prior to sonication, the surfaces on which biofilm is grown are rinsed thoroughly to remove all planktonic bacteria. Rinsing is enough to wash off free floating planktonic bacteria.

Day 0

1. Bacteria inocula, *Staphylococcus aureus*, (ATCC: 6538), (at 1 x 10^8 cfu/ml) were diluted 1:10 into 4 ml saline, and further diluted 1:100 into M103 media (M103 media filter sterilized: 1% Serum, 0.25% Glucose, 0.1% Neopeptone) for starting M103 inocula of approximately 1 x 10^5 cfu/ml (t=0).

2. T=0 inocula of the 1 x 10^5 cfu/ml were plated on TSA at 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} dilutions, and plates were incubated overnight at 35° C.

3. 6 (3 treated and 3untreated) Luer activated devices as above were used. 2ml saline was injected into each device, and each device was actuated 25 times to simulate actual use.

4. 2 ml of M103 inoculum (containing 1 x 10^5 cfu/ml bacteria) were pushed through each device, and devices with the same treatment conditions were placed in 50ml conical tubes.

5. Tubes containing the devices were incubated overnight at 35° C.

Day 1

1. T=0 plates were counted.

2. 2 ml of sterilized M103 media with a 10^3 dilution of bacteria was pushed through each device. This step was continued for 6 successive days. Each device was actuated 25 times per day to simulate actual use for a total of 175 actuations for samples in 168h test.

Day 7

1. To remove any non-adherent bacteria, 10 ml of saline + 0.1% Tween 80 were pushed through each device.

2. To ensure only pure saline was in the device for sonication, 2 ml saline was pushed through each device.

3. All duplicate devices were placed in a 16 x 125 mm glass test tube.

4. All tubes are placed in a room temperature water bath in the sonicator. The water in the bath was covering the heights of the devices in the tubes.

5. The tubes were sonicated for 1 min, and rested for 1 min, alternating for 5 total times.

6. 1 ml saline was passed through all devices, and collected in a 24 well plate.

7. 100 μl was pipetted from the collected flow-through for each device into the well of the first row of a 96 well-micro titer plate. 180 μl of 0.9% sterile saline is added in the wells down each column.

8. A serial ten fold dilution (100-10^{-4}) was prepared down each of the rows by transferring 20 μl from each dilution well.

9. 100μl samples from the wells with 10^{-1}, 10^{-3}, and 10^{-5} dilutions were transferred to TSA plates and spread for counting, for final dilutions of 10^{-2}, 10^{-4}, and 10^{-6}.

10. Incubated at 35 °C overnight and the plates were counted. Results follow.

TABLE 18 T=0 plate count

Dilution	Plate count: A	Plate count B
(-3)	152	224
(-4)	20	17
(-5)	1	1
(-6)	0	0

Starting CFU of M103 bacteria: **1.58 x 10^5**

Table 19: Plate Count after 7 Days

Dilution	Treated Sample 1	Treated Sample 2	Treated Sample 3	Untreated Sample 1	Untreated Sample 2	Untreated Sample 3
(-1)	1	0	0	TMTC	TMTC	TMTC
(-3)	0	0	0	792	774	984
(-5)	0	0	0	26	20	12
(-7)	-	-	-	1	0	5

TMTC= too many to count

Table20: Average CFU and Log Reduction in Silver Nanoparticle Coated Device

	Treated	Untreated
Ave. CFU	3.33	$8.50*10^5$
Log CFU	0.52	5.93
Log Red	5.41	-

The results show that silver nanoparticle treated Luer activated device having polycarbonate and silicone surfaces exhibited strong inhibition of biofilm formation for 7 days. The quantitative measure, the log reduction in bacterial count compared to an untreated device is > 5 log translating into a 99.999% reduction.

Example 96 Preparation of gold nanoparticles

In a test tube, sodium oleate solution (0.125M, 1mL), aqueous hydrogen tetrachloroaurate trihydrate (1% w/v, 1 mL) and disodium EDTA solution (0.125M, 0.2 mL) were added in succession. The test tube was placed in microwave oven and heated briefly to increase solution temperature to ~ 45-50°C (color change to blue black seen) and the test tube left to cool to room temperature under lab light. After 4h, the blue black color had changed to wine red and color became much darker. The solution remained red color for over a month at ambient temperature. The UV/VIS absorption peak was around 530 nm.

Example 97 Gold nanoparticles preparation – Method - 2

In a test tube, following solutions & chemicals were added and tube heated briefly as described in example 97.

Sodium oleate solution (0.125M, 0.9 mL)

Hydrogen tetrachloroaurate trihydrate (1% w/v, 0.1 mL)

De-ionized water (0.9 mL)

Disodium EDTA solution (0.125M, 0.1 mL)

The color of tube contents changed to pale yellow and then to wine red. No precipitation was observed and wavelength maximum was 530 nm.

Example 98 Gold nanoparticles preparation – Method – 3

In a test tube, sodium oleate solution (0.125M, 1mL), hydrogen tetrachloroaurate trihydrate (1% w/v, 1 mL) and TEMED (0.1 mL) were added in succession. The yellow colored solution changed in intensity after TEMED addition. The test tube was placed in microwave oven and heated briefly to increase solution temperature to ~ 45-50° C (color change to yellow brown seen) and then as the test tube cooled to room temperature it finally turned red in color. After 4h, the blue black color had changed to wine red and color intensity dark. No agglomeration of particles in solution was seen. The UV/VIS absorption peak was around 530 nm.

Example 99 Gold nanoparticles preparation – Method – 4

This example was carried out like example 99 except instead of sodium oleate as stabilizer we used Novec® 4430 (a fluorinated surfactant from 3M Company) solution (32g/L). A clear violet purple solution was obtained having wavelength maximum at ~ 580 nm.

These examples describe the preparation of gold nanoparticles using methods of the present invention. In these methods, such as where noble metals such as gold, copper, rhodium, platinum or palladium are used, the use of an anion compound may be optional. In addition, reducing agents such as sodium borate, hydrazine hydrate, primary amines, lithium aluminum hydride and others known to those skilled in the art may be used to initiate nanoparticle synthesis.

Suitable stabilizers for such nanoparticles synthesis include polyacrylamide, carboxymethyl cellulose, TritonX-100®, T-MAZ®, Span 80®, Novec 4430, Novec 4432, PVA, PVP, polyurethane diol, sodium dodecyl sulfate, dioctyl sulfosuccinate, propylene glycol alginate, tartaric acid. Suitable initiators or reducing agents for such nanoparticles may be TEMED, triethanolamine (TEA) and TEA-water mixtures (0.1 to 90% TEA), tetrabutyldiamine and its aqueous solution (0.1 to 90% amine), tetradimethyldiaminomethane and its aqueous solution (0.1 to 90% organic moiety), aldehydes such as formaldehyde, glutaraldehyde, and dipropylamine and its aqueous mixtures (0.1 to 90% amine). A suitable gold compound may be hydrogen tetrachloroaurate trihydrate, but other gold compounds if available may be used without departing from the scope of the invention.

CLAIMS

What is claimed is:

What is claimed is:

1. A method of rendering an elastomeric surface electrically conductive, comprising,

a) contacting an elastomeric surface with a solution comprising metal nanoparticles for a time sufficient for an effective amount of the nanoparticles to adhere to the surface, and

b) rinsing the surface.

2. The method of Claim 1, wherein the metal nanoparticles are made by a method comprising,

a) adding in no particular order, an aqueous solution of a stabilizing agent solution, an anionic donating solution and a soluble metal salt solution, and

b) adding a reducing solution.

3. The method of Claim 1 wherein the metal nanoparticle comprises silver, gold, platinum, iridium, rhodium, palladium, copper or zinc.

4. The method of Claim 2, further comprising, c) heating the final solution.

5. The method of Claim 1, wherein the contacting and rinsing steps are repeated multiple times to increase the number of nanoparticles adhering to the surface.

6. The method of Claim 1, wherein the surface contacted is silicone, polyurethane, synthetic or natural rubber, a synthetic or natural polymer, flexible polymers of polyimides, polyamides, polyacetals, polysulfones, PBTs, PBO's, ethylene and propylene based polymers, acetate polymers, polyacrylates, polycarbonate, PET's, PEN's or blends thereof or co-polymeric derivatives.

7. The method of Claim 1, further comprising

c) contacting the elastomeric surface with nanoparticles adhered thereto with an aqueous solution of hydrogen peroxide for a sufficient period of time, and

d) rinsing the hydrogen peroxide solution from the surface.

8. The method of Claim 7, wherein the surface contacted is silicone, polyurethane, synthetic or natural rubber, a synthetic or natural polymer, flexible polymers of polyimides, polyamides, polyacetals, polysulfones, PBTs, PBO's, ethylene and propylene based polymers, acetate polymers, polyacrylates, polycarbonate, PET's, PEN's or blends thereof or co-polymeric derivatives.

9. An article produced by a method of rendering an elastomeric surface electrically conductive, wherein the method comprises

a) contacting an elastomeric surface with a solution comprising metal nanoparticles for a time sufficient for an effective amount of the nanoparticles to adhere to the surface, and

b) rinsing the surface.

10. The article of Claim 9, comprising flexible mirrors, stretchable elastic conductive polymers, articles used to reduce electromagnetic interference, to shield devices and circuits against electrostatic discharging, and to impart radar invisibility to aircraft or other vehicles.

11. A method of rendering an article or surface contacting a fluid resistant to biofilm formation, comprising,

a) contacting the article or surface with a solution comprising metal nanoparticles for a time sufficient for an effective amount of the nanoparticles to adhere to the surface, and

b) rinsing the surface.

12. The method of Claim 11, wherein the metal nanoparticles are made by a method comprising,

a) adding in no particular order, an aqueous solution of a stabilizing agent solution, an anionic donating solution and a soluble metal salt solution, and

b) adding a reducing solution.

13. The method of Claim 11 wherein the metal nanoparticle comprises silver, gold, platinum, iridium, rhodium, palladium, copper or zinc.

14. The method of Claim 12, further comprising, c) heating the final solution.

15. The method of Claim 11, wherein the contacting and rinsing steps are repeated multiple times to increase the number of nanoparticles adhering to the surface.

16. The method of Claim 11, wherein an article or surface contacting a fluid that is contacted by the nanoparticles is made of steel, stainless steel, glass, titanium, copper, gold, synthetic and natural polymers, polypropylene, polycarbonate, polyurethane, polyvinyl chloride, polystyrene, polysulfone, silicones, HTV, RTV, blends or co-polymeric derivatives.

17. The method of Claim 11, further comprising

c) contacting the elastomeric surface with nanoparticles adhered thereto with an aqueous solution of hydrogen peroxide for a sufficient period of time, and

d) rinsing the hydrogen peroxide solution from the surface.

18. The method of Claim 17, wherein an article or surface contacting a fluid that is contacted by the nanoparticles is made of steel, stainless steel, glass, titanium, copper, gold, synthetic and natural polymers, polypropylene, polycarbonate, polyurethane, polyvinyl chloride, polystyrene, polysulfone, silicones, HTV, RTV, blends or co-polymeric derivatives.

19. An article produced by rendering an article or surface contacting a fluid resistant to biofilm formation, wherein the method comprises

a) contacting an article or surface contacting a fluid with a solution comprising metal nanoparticles for a time sufficient for an effective amount of the nanoparticles to adhere to the article or surface, and

b) rinsing the article or surface.

20. The article of Claim 19, comprising food storage and preparation devices, laboratory equipment, marine or water vehicles, hulls, propellers, anchors, ballast tanks, motors, pilings,

liquid filtering equipment, tubing, ropes, chains, fish tanks, liquid containers, water bowls, cooling towers, water tanks, canteens, fuel tanks, or storage bins.

21. A method of making metal nanoparticles comprising,

a) adding in no particular order, an aqueous solution of a stabilizing agent solution, an anionic donating solution and a soluble metal salt solution, and

b) adding a reducing solution.

22. The method of Claim 21 wherein the metal comprises silver, gold, platinum, iridium, rhodium, palladium, copper or zinc.

23. The method of Claim 21, further comprising, c) heating the final solution.

24. The method of Claim 21, wherein the stabilizing agent solution comprises a surfactant, a polymer or both.

25. The method of Claim 24, wherein the polymer is a homopolymer copolymer, synthetic or naturally derived, polymers of acrylamide and its derivatives, methacrylamide and its derivatives, polyamides, polyurethanes, polymers having no particular backbone but with urethane segments or tertiary amine groups in the side chains, other polymers predominantly polar in nature or co-polymers having a portion that is derived from polar co-monomers, methaacrylamide, substituted acrylamides, substituted methaacrylamides, acrylic acid, methacrylic acid, hydroxyethyl methacrylate, acrylonitrile, 2-acrylamido-2-methylpropane sulfonic acid and its salts (sodium, potassium, ammonium), 2-vinyl pyrrolidone, 2-vinyl oxazoline, vinyl acetate, maleic anhydride.

26. The method of Claim 21, further comprising, forming the nanoparticles in situ on the surface of an article.

27. The method of Claim 21, further comprising, extracting the nanoparticles into a non-aqueous solution.

28. A metal nanoparticle made by a method of

a) adding in no particular order, an aqueous solution of a stabilizing agent solution, an anionic donating solution and a soluble metal salt solution, and

b) adding a tertiary diamine solution.

29. The metal nanoparticle of Claim 28, wherein the metal comprises silver, gold, platinum, iridium, rhodium, palladium, copper or zinc.

30. The metal nanoparticle of Claim 28, further comprising, c) heating the final solution.

31. The metal nanoparticle of Claim 28, wherein the stabilizing agent is a polymer, a surfactant or both.

32. The metal nanoparticle of Claim 28, wherein the polymer is a homopolymer copolymer, synthetic or naturally derived, acrylamide and its derivatives, methacrylamide and its derivatives, polyamides, polyurethanes, polymers having no particular backbone but with urethane segments or tertiary amine groups in the side chains, other polymers predominantly polar in nature or co-polymers having a portion that is derived from polar co-monomers, methaacrylamide, substituted acrylamides, substituted methaacrylamides, acrylic acid, methacrylic acid, hydroxyethyl methacrylate, acrylonitrile, 2-acrylamido-2-methylpropane sulfonic acid and its salts (sodium, potassium, ammonium), 2-vinyl pyrrolidone, 2-vinyl oxazoline, vinyl acetate, maleic anhydride.

33. The metal nanoparticle of Claim 28, wherein the surfactant is an anionic, nonionic, or amphoteric surfactant.

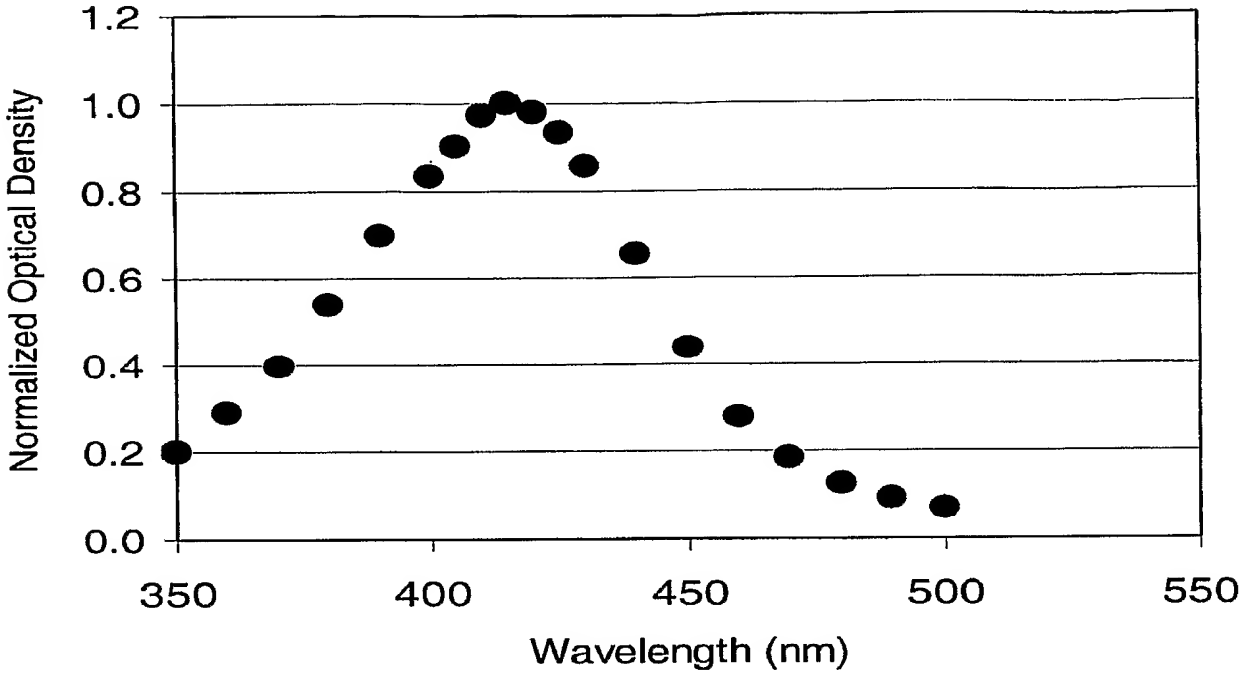

Normalized Optical Density
1.2
1.0
0.8
0.6
0.4
0.2
0.0
350
400
450
500
550
Wavelength (nm)

Fig. 1

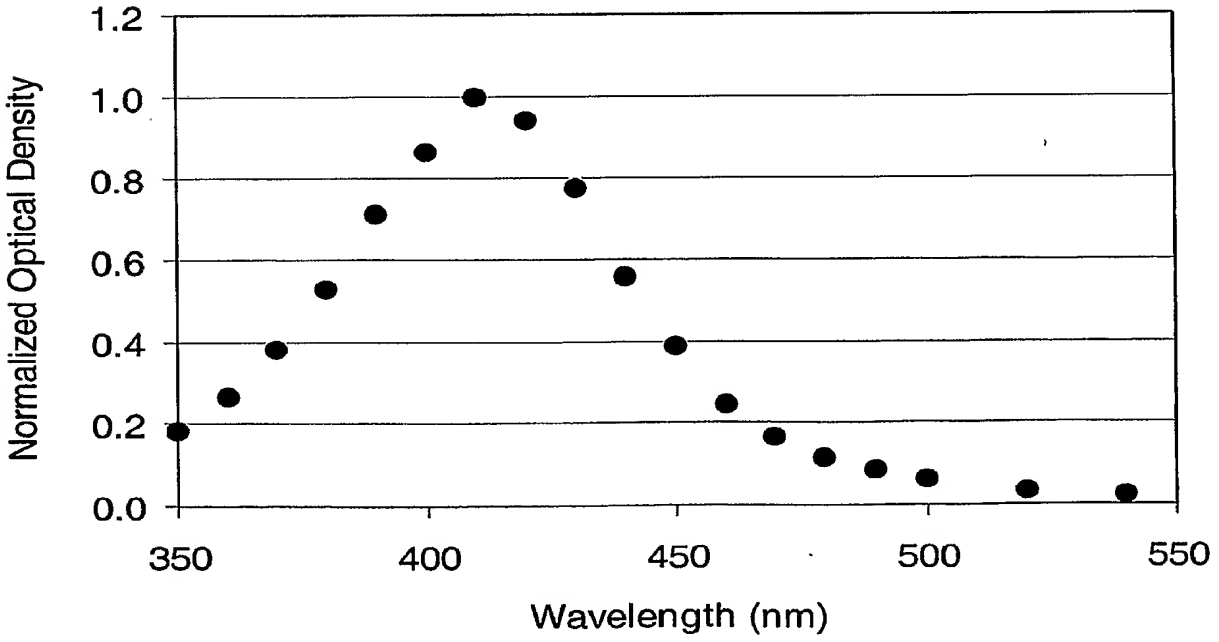

Normalized Optical Density
1.2
1.0
0.8
0.6
0.4
0.2
0.0
350
400
450
500
550
Wavelength (nm)

Fig. 2

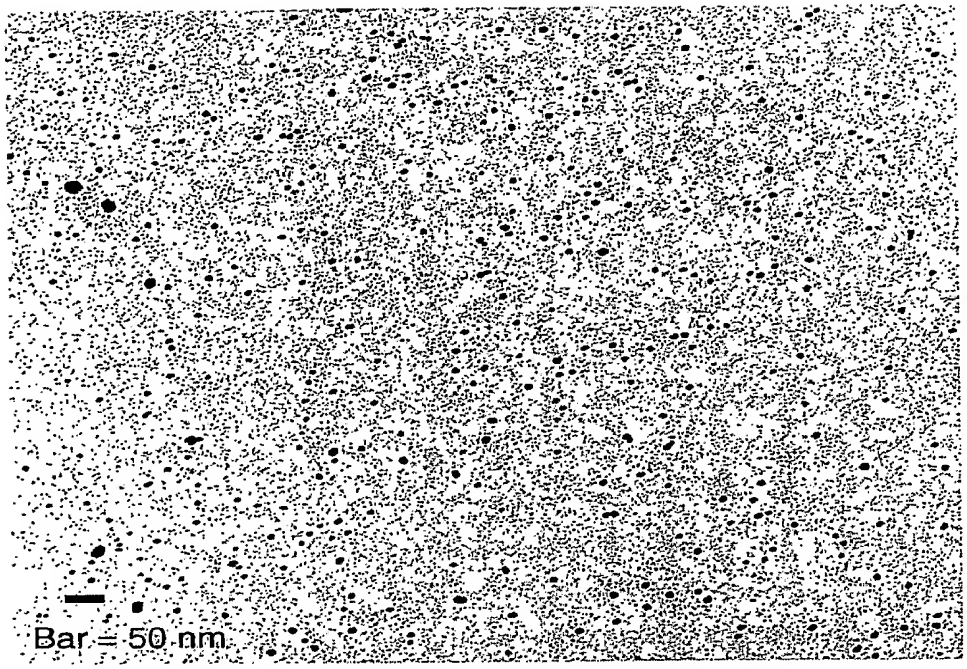

Bar = 50 nm

Fig. 3

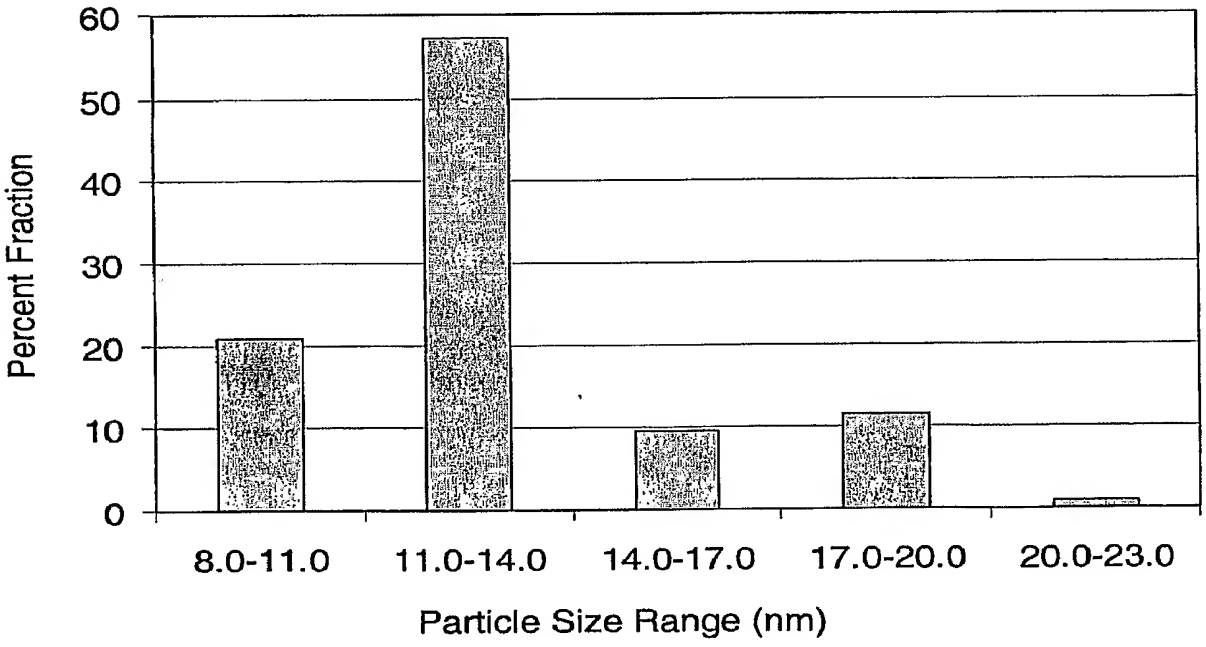

60
50
40
30
20
10
0
Percent Fraction
8.0-11.0
11.0-14.0
14.0-17.0
17.0-20.0
20.0-23.0
Particle Size Range (nm)

Fig. 4

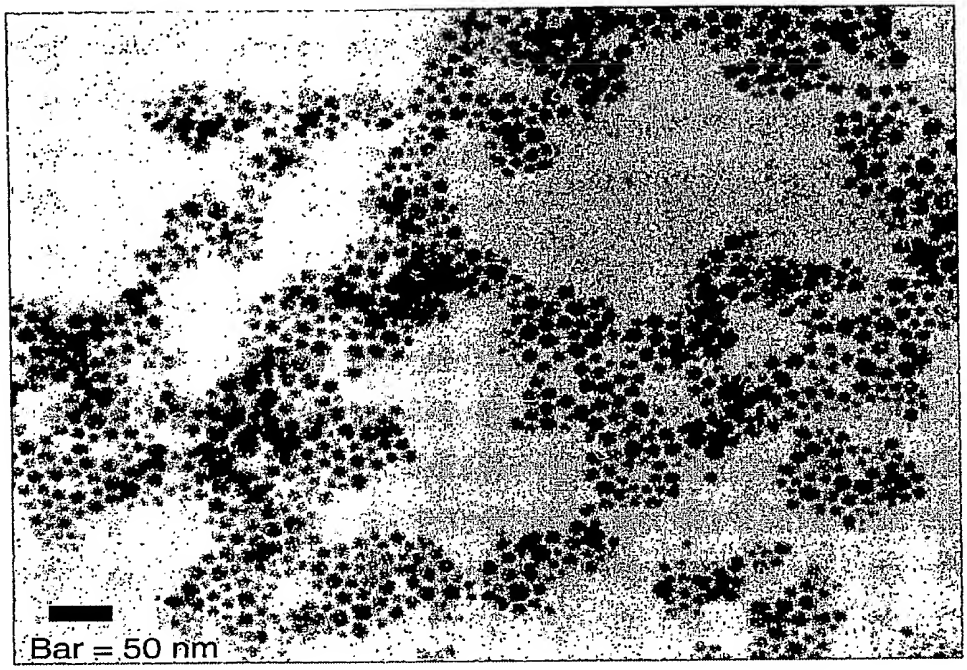

Bar = 50 nm

Fig. 5

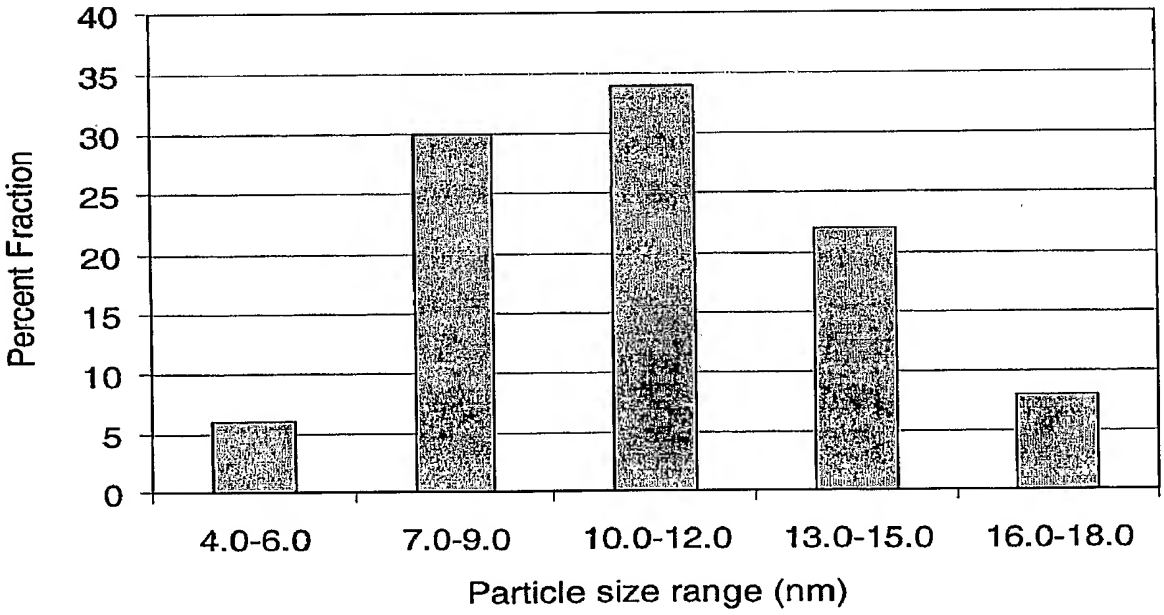

Percent Fraction
40
35
30
25
20
15
10
5
0
4.0-6.0
7.0-9.0
10.0-12.0
13.0-15.0
16.0-18.0
Particle size range (nm)

Fig. 6

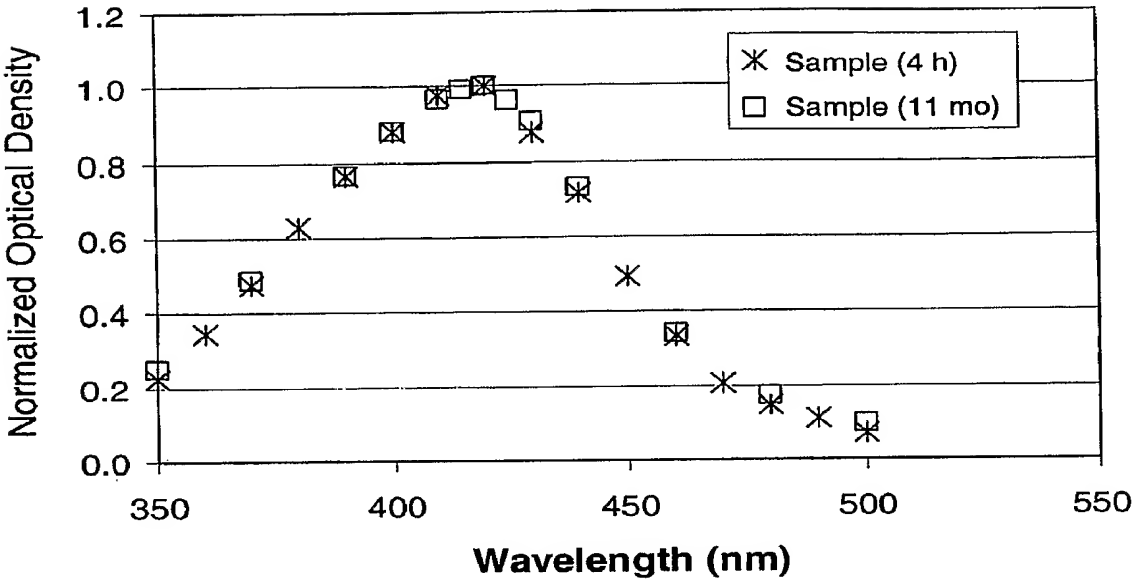

Normalized Optical Density
1.2
1.0
0.8
0.6
0.4
0.2
0.0
350
400
450
500
550
Wavelength (nm)
Sample (4 h)
Sample (11 mo)

Fig. 7

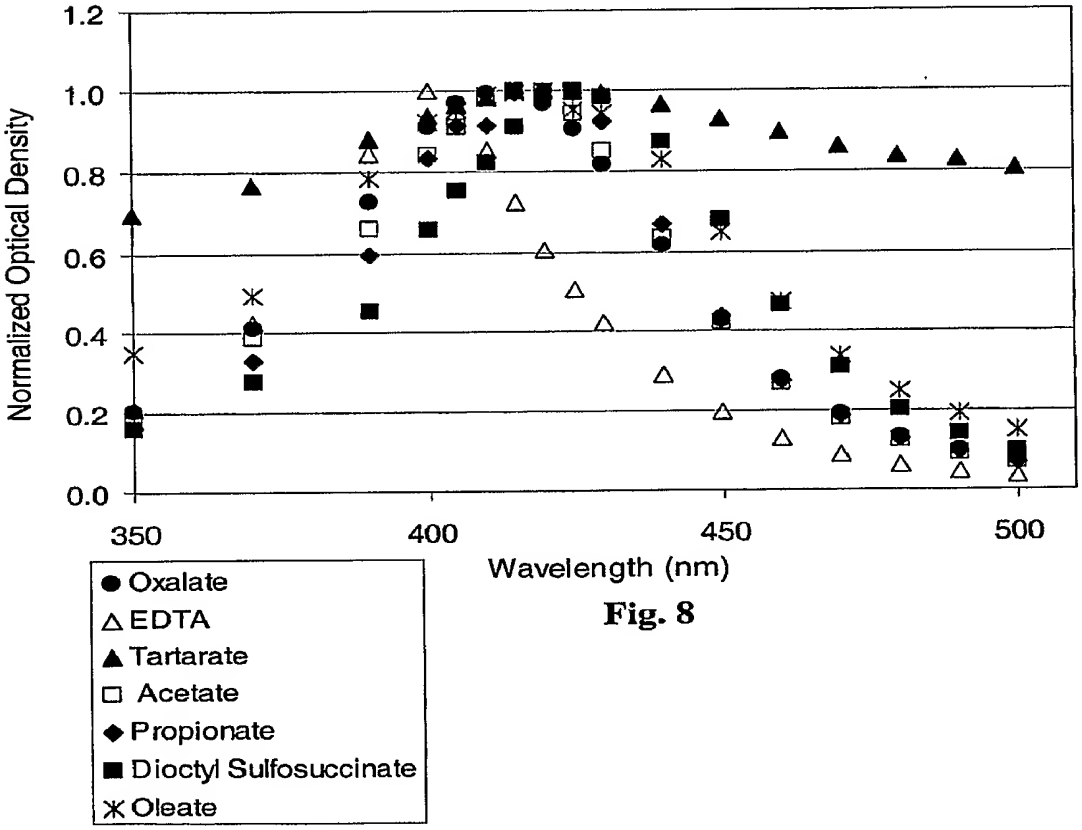

Normalized Optical Density
1.2
1.0
0.8
0.6
0.4
0.2
0.0
350
400
450
500
Wavelength (nm)
Oxalate
EDTA
Tartarate
Acetate
Propionate
Dioctyl Sulfosuccinate
Oleate

Fig. 8

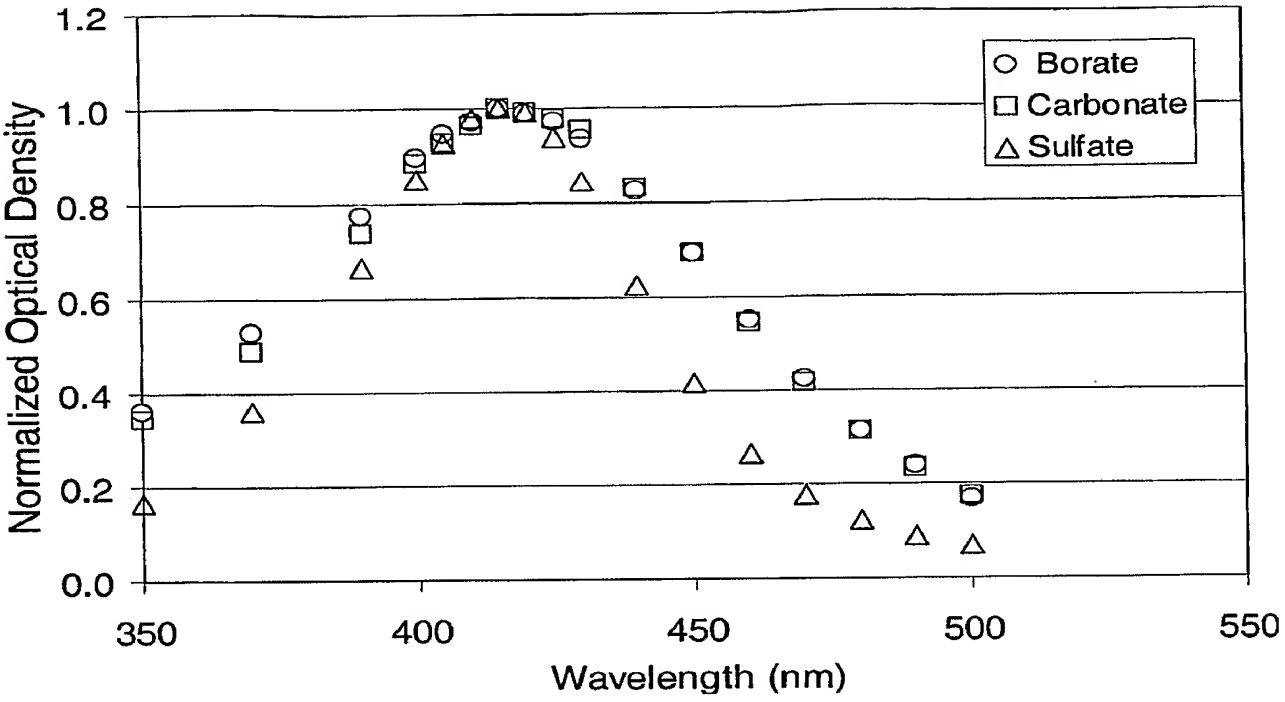

Normalized Optical Density
1.2
1.0
0.8
0.6
0.4
0.2
0.0
350
400
450
500
550
Wavelength (nm)
Borate
Carbonate
Sulfate

Fig. 9

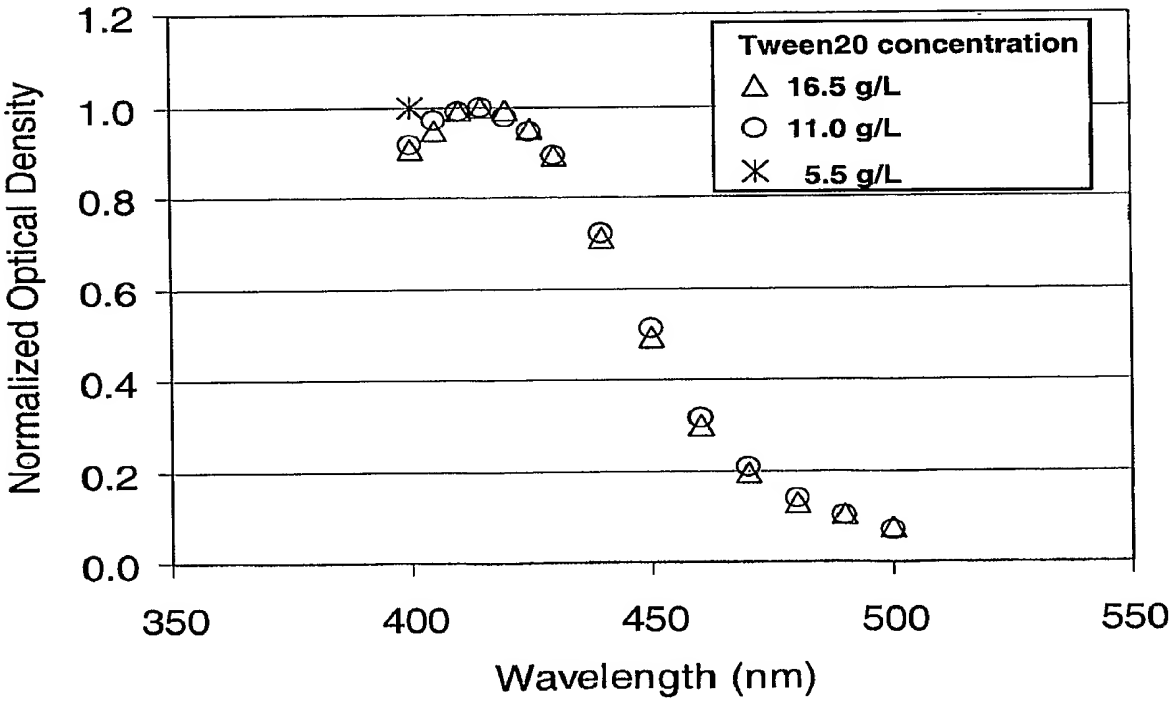

Normalized Optical Density
1.2
1.0
0.8
0.6
0.4
0.2
0.0
350
400
450
500
550
Wavelength (nm)
Tween20 concentration
16.5 g/L
11.0 g/L
5.5 g/L

Fig. 10

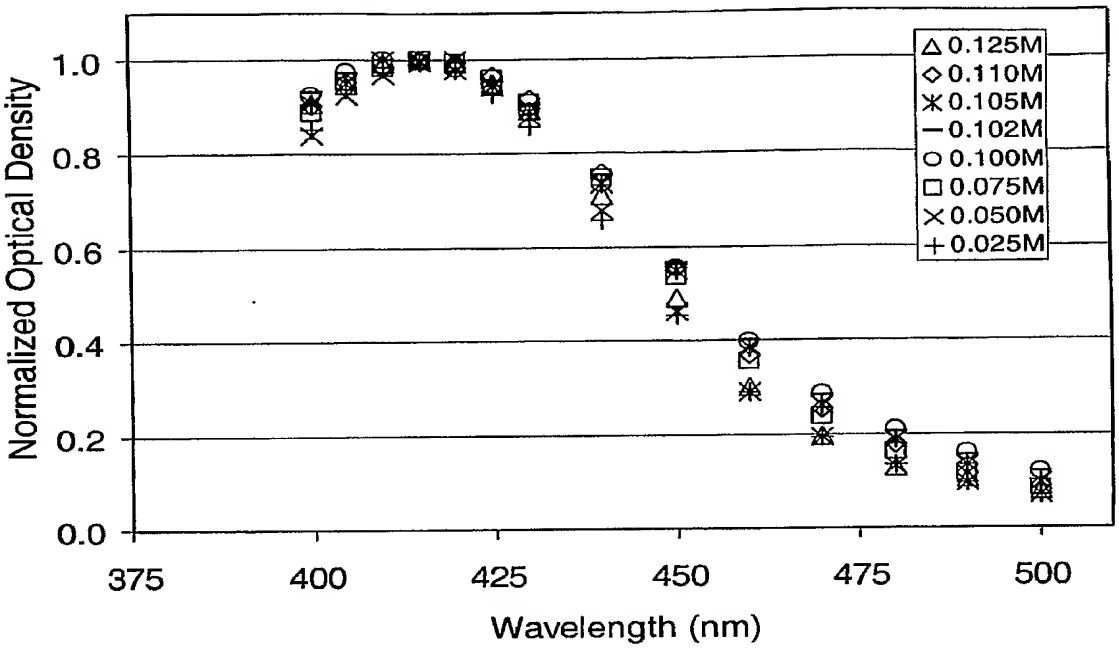

Normalized Optical Density
1.0
0.8
0.6
0.4
0.2
0.0
375
400
425
450
475
500
Wavelength (nm)
0.125M
0.110M
0.105M
0.102M
0.100M
0.075M
0.050M
0.025M

Fig. 11

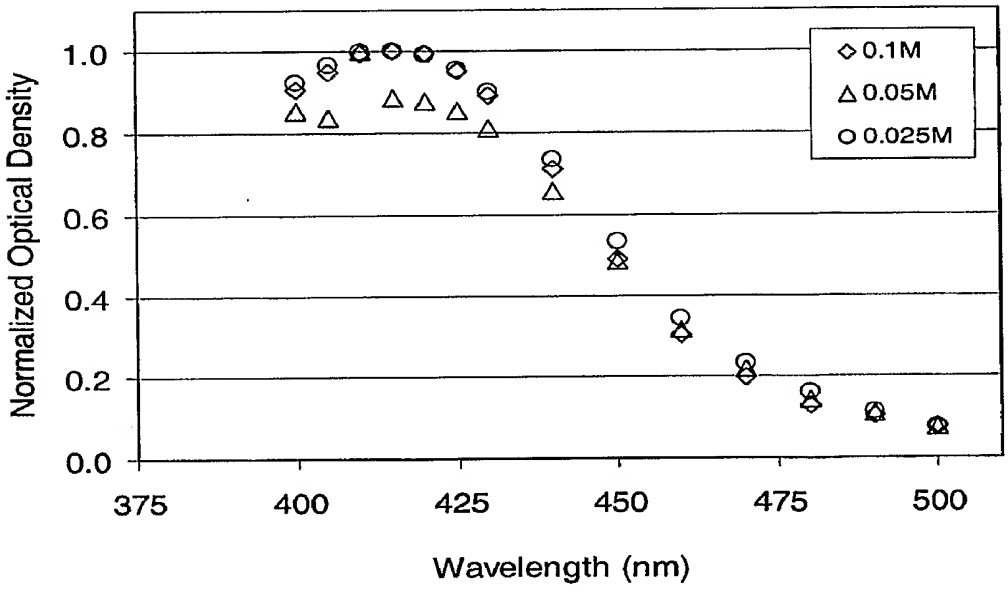

Normalized Optical Density
1.0
0.8
0.6
0.4
0.2
0.0
375
400
425
450
475
500
Wavelength (nm)
0.1M
0.05M
0.025M

Fig. 12

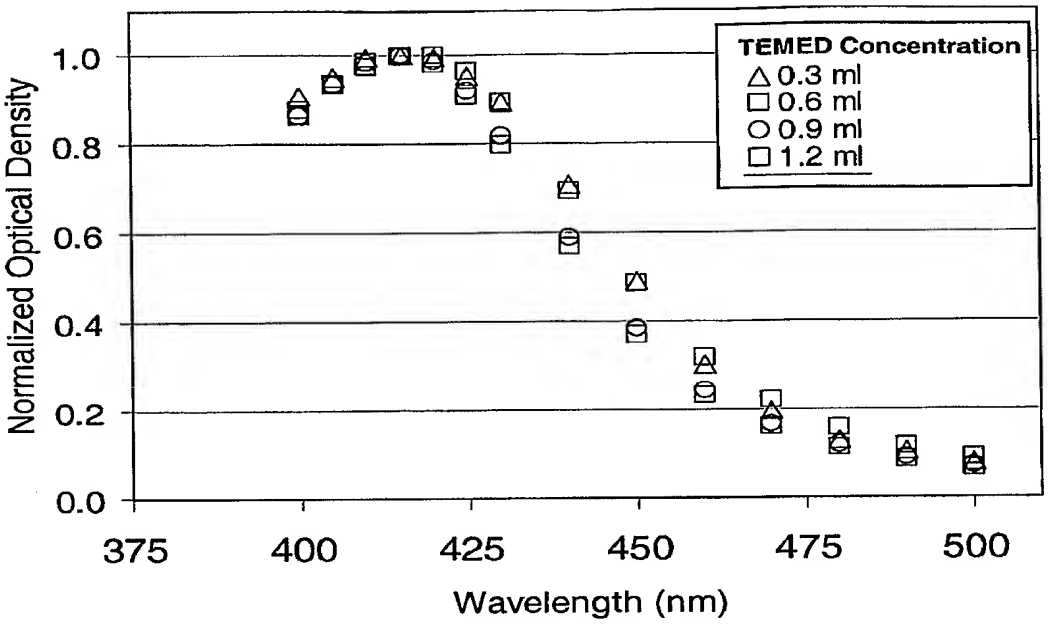

TEMED Concentration
0.3 ml
0.6 ml
0.9 ml
1.2 ml
Normalized Optical Density
1.0
0.8
0.6
0.4
0.2
0.0
375
400
425
450
475
500
Wavelength (nm)

Fig. 13

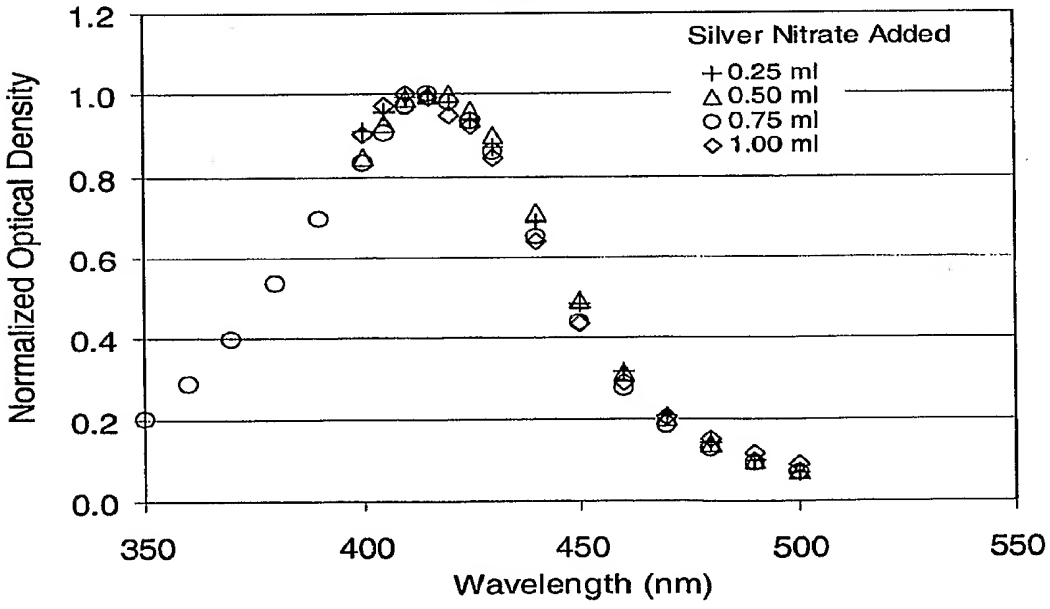

Silver Nitrate Added
0.25 ml
0.50 ml
0.75 ml
1.00 ml
Normalized Optical Density
1.2
1.0
0.8
0.6
0.4
0.2
0.0
350
400
450
500
550
Wavelength (nm)

Fig. 14

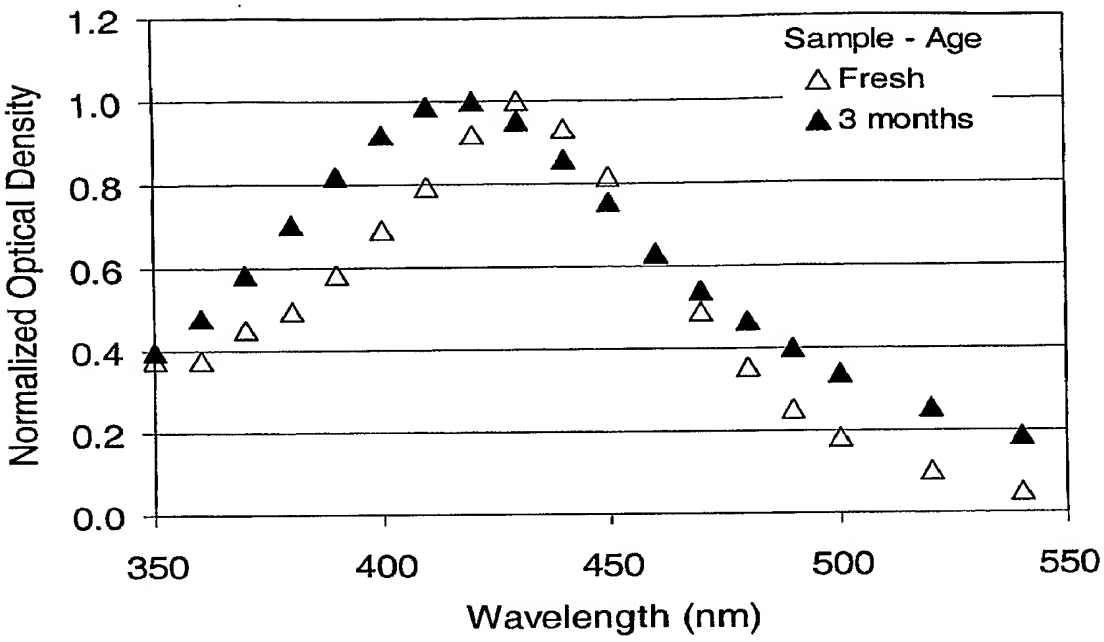

Normalized Optical Density
1.2
1.0
0.8
0.6
0.4
0.2
0.0
350
400
450
500
550
Wavelength (nm)
Sample - Age
Fresh
3 months

Fig. 15

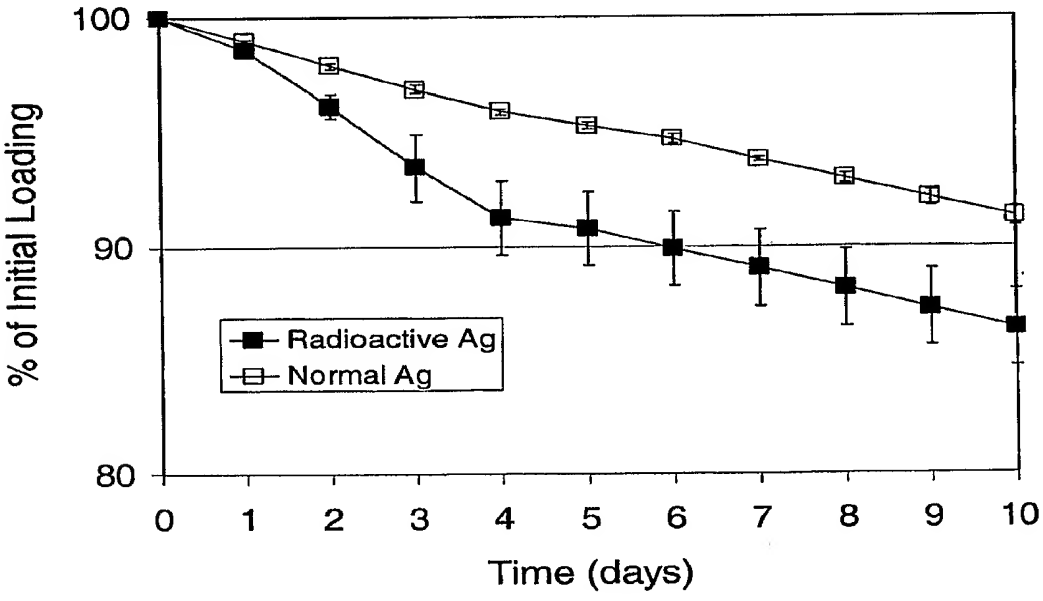

% of Initial Loading
100
90
80
0
1
2
3
4
5
6
7
8
9
10
Time (days)
Radioactive Ag
Normal Ag

Fig. 16

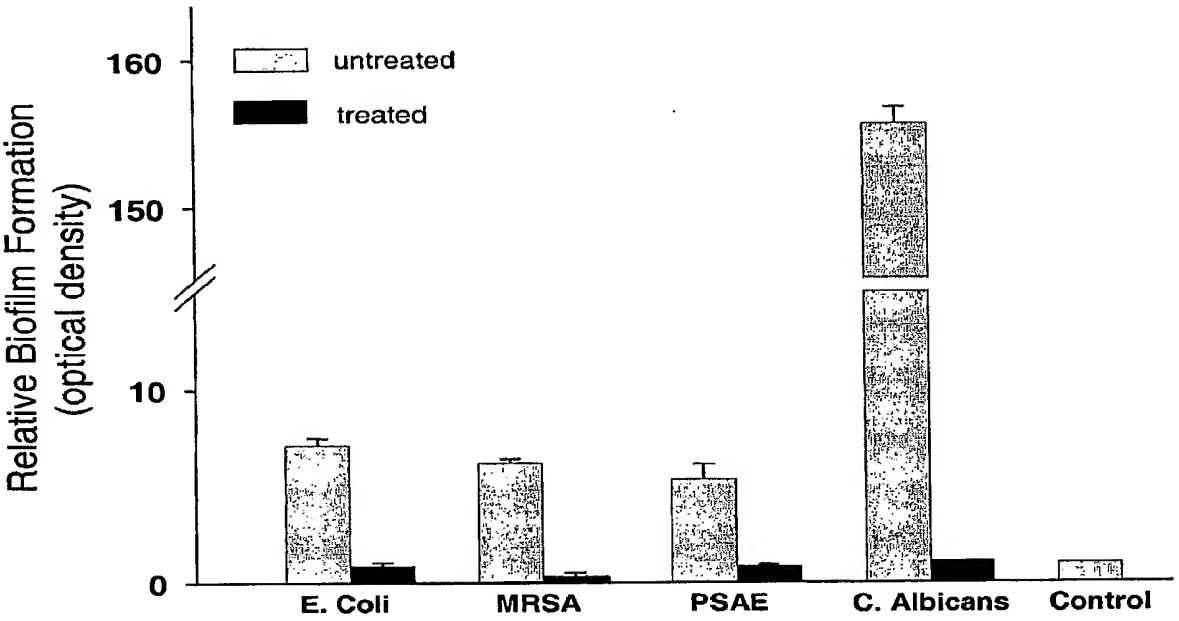

Relative Biofilm Formation
(optical density)
160
150
10
0
untreated
treated
E. Coli
MRSA
PSAE
C. Albicans
Control

Fig. 17

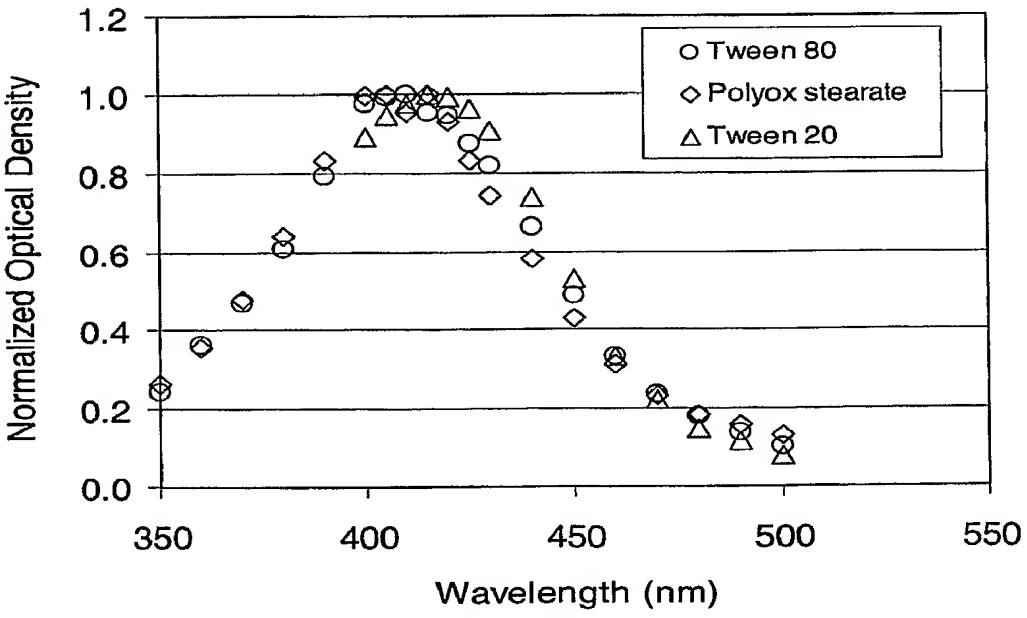

Normalized Optical Density
1.2
1.0
0.8
0.6
0.4
0.2
0.0
350
400
450
500
550
Wavelength (nm)
Tween 80
Polyox stearate
Tween 20

Fig. 18